STUDIES ON THE EFFECTS OF VARIOUS FUNCTIONAL GROUPS ON THE NORTHERN HEMISPHERE OF BRYOSTATIN THROUGH SYNTHESIS

by

Arnab Rudra

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry

The University of Utah

May 2014

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Arnab Rudra**

has been approved by the following supervisory committee members:

Gary E. Keck	, Chair	**10/11/2013** Date Approved
Janis Louie	, Member	**10/11/2013** Date Approved
Ryan E. Looper	, Member	**10/11/2013** Date Approved
Richard D. Ernst	, Member	**10/11/2013** Date Approved
Matthew Topham	, Member	**10/11/2013** Date Approved

and by **Cynthia J. Burrows**, Chair of

the Department of **Chemistry**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Bryostatin 1 is a complex natural product that was originally isolated by Pettit from the bryozoan *Bugula neritina*. Its intriguing structural complexity and remarkable biological profile against several human diseases such as cancer, Alzheimer's disease, HIV, and stroke have put bryostatin 1 in the forefront of research. The mechanism by which bryostatin mediates these effects are thought to be related to its ability to activate the protein kinase C (PKC) family of signaling enzymes. However, bryostatin is unique compared to most other known PKC activators such as the phorbol esters, as it is not a tumor promoter. The reasons behind bryostatin's distinct biological profile remain unknown and are of great interest for the development new drug leads that target PKC.

The work presented in this dissertation deals with the synthesis and biological evaluation of bryostatin 1 analogues modified in the northern hemisphere of the molecule. Specifically, the role of various substituents in the A and B ring region has been investigated by synthesizing Merle 30, 32, 34, and 38. This study suggested that these functional groups do not by themselves serve as functional switches between the PMA versus bryostatin 1-like activity of bryostatin analogues. Analogues with more polar groups in the A, B ring region tend to behave like bryostatin 1 as opposed to PMA. However, analogues with similar polarity in the northern hemisphere to that of bryostatin may not necessarily follow this trend as demonstrated via studies on Merle 34 and 38.

Dedicated to my mother Sipra Rudra, my father Saikat Kumar Rudra, my wife Kusumika Mukherjee, and my entire family

TABLE OF CONTENTS

STANDARD LIST OF ABBREVIATIONS

$[\alpha]^{20}_{D}$	specific rotation
Ac	acetyl
AcOH	acetic acid
AIDS	acquired immunodeficiency syndrome
$AlMe_3$	trimethyl aluminum
BF_3•OEt_2	boron trifluoride diethyletherate
BH_3•DMS	borane dimethylsulfide complex
BINOL	(1,1-binapthalene)-2,2-diol
BITIP	catalyst made by combining (1,1-binapthalene)-2,2-diol and Ti(O*i*-Pr)$_4$
Bn	benzyl
BOM	benzyloxy methyl
BPS	*tert*-butyldiphenylsilyl
*n*Bu	butyl
*t*Bu	*tert*-butyl
*n*BuLi	*n*-butyllithium
Bu_3B	tributylborane
Bz	benzoyl
°C	degrees Celsius

clogP	computational log of partition coefficient
CAA	catalytic asymmetric allylation
calcd	calculated
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CH_2Cl_2	dichloromethane
COSY	correlation spectroscopy
CSA	10-camphorsulfonic acid
d	day(s); doublet (spectral)
DCC	1,3-dicyclohexylcarbodiimide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene,
DDQ	2,3-dicyano-5,6-dichloro-parabenzoquinone
de	diastereomeric excess
dr	diastereomeric ratio
DEPT	distortionless enhancement by polarization transfer
DIBALH	diisobutylaluminun hydride
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMP	dimethoxypropane
DMS	dimethylsulfide
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
ee	enantiomeric excess

er	enantiomeric ratio
equiv	equivalent(s)
Et	ethyl
EtOH	ethanol
Et_2O	diethylether
EtOAc	ethyl acetate
EI	electron impact
ESI	Electrospray ionization
FAB	fast atom bombardment
g	gram(s)
h	hour(s)
HF•py	hydrogen fluoride pyridine complex
HMDS	hexamethyldisilazane
HRMS	high-resolution mass spectrum
Hz	hertz
IC_{50}	50% inhibitory concentration
IR	infrared
J	coupling constant (in NMR)
K_i	dissociation constant
LAH	lithium aluminum hydride
LDA	lithium diisopropyl amide
LiHMDS	lithium hexamethyldisilazide
LRMS	low-resolution mass spectrum

M	molarity, mol/L
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
Me_2AlCl	dimethylaluminum chloride
MeCN	acetonitrile
MeOH	methanol
MHz	megahertz
min	minute(s)
mL	milliliter
MMPP	magnesium monoperoxyphthalate
MNBA	2-methyl-6-nitrobenzoic anhydride
mol	mole(s)
mp	melting point
MS	mass spectrometry; molecular sieves
Ms	methanesulfonyl
NaH	sodium hydride
NaHMDS	sodium hexamethyldisilazide
NMM	*N*-methylmorpholine
NMO	*N*-methylmorpholine-N-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy
Ph	phenyl

PKC	protein kinase C
PMB	*para*-methoxybenzyl
$P(OEt)_3$	triethylphosphite
ppm	parts per million (in NMR)
PPTs	pyridinium p-toluenesulfonate
*i*Pr	isopropyl
$(iPr)_2NH$	diisopropylamine
py	pyridine
q	quartet (spectral)
quant	quantitative
R_f	retention factor (in chromatography)
rb	round bottom
RCM	Ring-Closing Metathesis
rt	room temperature
s	singlet (spectral); second(s)
sp.	species
SO_3•py	sulfur trioxide pyridine complex
t	triplet (spectral)
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxyl
TES	triethylsilyl
Tf	trifluoromethanesulfonyl triflate

TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TNFα	tumor necrosis factor alpha
TPAP	tetrapropylammonium perruthenate
Ts	*p*-toluenesulfonyl, tosic
TsOH	*p*-toluenesulfonic acid

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank all of them who made the completion of this work possible. At first, I would like to thank my research advisor, boss Keck, for his guidance and constant support. His deep insight in organic chemistry, amazing ability to offer simple solutions to complex problems and above all brilliant style of teaching inspired us to pursue independent creative thinking throughout our graduate education. It has been a great privilege to have him as my mentor.

I would also like to thank my committee members, Prof. Ryan Looper, Prof. Janis Louie, Prof. Richard Ernst, and Prof. Matthew Topham, for their support throughout my graduate study and critiquing this dissertation.

I am grateful to the people in the Keck group, past and present, for their friendship, support and cooperation. I would like to thank Dr. Lars Heumann and Dr. Matthew Kraft for their help during my first year in the Keck group. They sincerely taught me laboratory techniques needed for this dissertation work. I would like to thank Dr. Wei Li, Dr. Yam Poudel, Xiguang Zhao, Thomas Cummins, Mark Peterson, Dr. Sherry Chavez, and Dr. Bob Giles for their help on numerous occasions over the years. The presence of Dr. Heumann, Dr. Kraft, Dr. Li, Dr. Poudel, Xiguang, and Thomas made life in the laboratory a real fun. I would also like to thank Kevin McGowan, David Baumann, and Sharon Kirk and Mark Peterson for participating in my projects.

I would like to express my gratitude to Dr. Peter Flynn, Atta Aarif and Dennis

Edward for their help with NMR and Dr. Jim Muller for his help with mass spectrometry.

It would not have been possible for me to reach this far without the guidance of my teachers who taught me chemistry. I would like to take this opportunity to thank Dr. Chandrakanta Bandyopadhyay, Dr. Ranabir Sur, Dr. Ranjan Patra, Dr. Arunava Sen, Dr. Sripati Bhusan Chakraborty, Dr. Bholanath Mukherjee, Dr. Shubhabrata Banerjee, Dr. Ajay Misra, and Dr. Dipesh Ghosh, for their patience with me while I was in my undergraduate college.

I would like to thank my family members for their endless love and support. My parents, Sipra Rudra and Saikat Kumar Rudra, are my greatest inspiration for pursuing higher studies. I especially like to thank my wife, Dr. Kusumika Mukherjee, for her patience, support and love over the years. I am also grateful to my parents in law, Madhab Narayan Mukherjee and Krishna Mukherjee for their faith in me.

Finally, I must thank Tomissa Carr and Jo Hoovey for their constant support during my time in the graduate school.

CHAPTER 1

STUDIES ON THE EFFECT OF THE C9 HYDROXY GROUP AND THE C8 GEM-DIMETHYL GROUP ON THE BIOLOGICAL PROFILE OF BRYOSTATIN 1

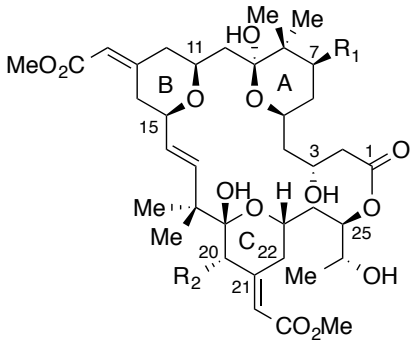
Introduction

Marine microbiology has recently become a popular area of research in pursuit of developing new drug candidates against various human ailments. In that context, marine bacterial species have gained tremendous attention due to their amazing ability to produce various small molecule natural products with varying degrees of structural complexities.[1] One of the examples of such biologically interesting natural products isolated from the marine environment is bryostatin 1. Bryostatin 1 is the most extensively studied member of some 20 structurally related polyketide macrolide natural products. In 1970, George Pettit and coworkers found that extracts from the marine bryozoan *Bugula neritina* from the Gulf of Mexico demonstrated antineoplastic properties.[2] The original source of these agents was later found to be the bacterial endosymbiont *Candidatus endobugula sertula*.[3] To date, 19 other members of the bryostatin family of natural products have been isolated.[4] It was not until several years later in 1982 that the structure of bryostatin 1 was elucidated by pioneering work of the Pettit group.[5] The same group in

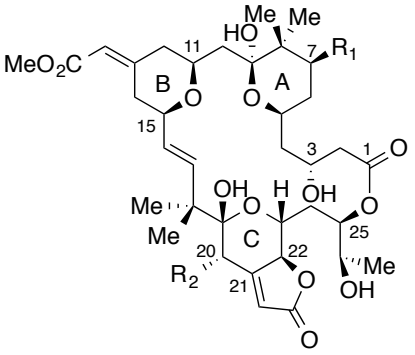
1991 confirmed the absolute configuration of bryostatins using heavy atom dispersion effects in the X-ray structure of a *p*-bromobenzoate derivative of bryostatin 2.[6] It was suggested that bryostatin acts as a deterrent against marine predators like fish and other organisms in protecting bryozoans; in return, the symbiotic bacteria gets a suitable environment to grow.[7] The structures of bryostatins 1-20 are summarized in Figure 1.1.

Structural features of the bryostatins

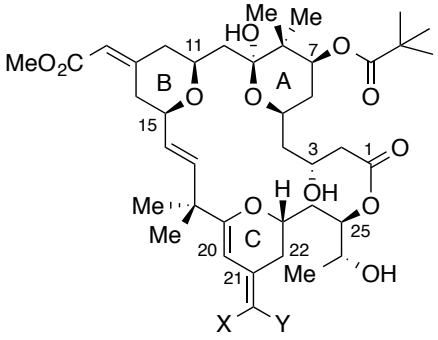
Structural complexities of the bryostatins have inspired organic chemists since their discovery. All the members of the bryostatin family are characterized by 20-membered macrolactones in which there are three highly substituted pyran rings embedded within the macrocycle; these are referred to as the A-, B-, and C-rings. The pyran rings are linked by a C16-C17 (*E*)-disubstituted olefin, *gem*-dimethyl functionality at C18, and a methylene bridge at C10. All the members also have a pair of geminal dimethyls at C8 and C18, as well as an exocyclic enoate at C13 and C21 in common. Besides their structural differences at C7 and C20, bryostatins 3, 19, and 20 have a fused butenolide to the C-ring. Bryostatins 10, 11, 13, 18, and 20 are all missing C20 substitution and bryostatins 16 and 17 have a simplified C-ring in the form of a glycal. Bryostatins 17 and 18 have opposite methyl enoate geometry in the C-ring. Interestingly, neristatin 1, although not included in the bryostatin family, has striking similarity in structure with the members in the family.[8] The crystal structure of bryostatin 1 solved by the Clardy group indicated the presence of an intramolecular transannular hydrogen-bonding network between the C19 hydroxyl hydrogen atom and the C3 hydroxyl oxygen as well as between the C3 hydroxyl hydrogen and the A- and B-ring pyran oxygens.[5]



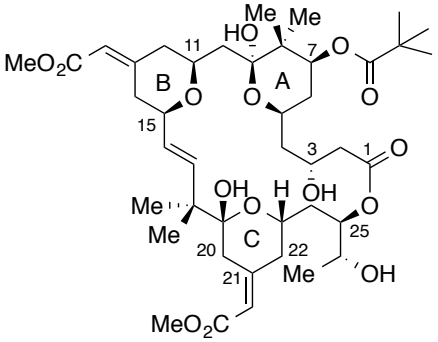
R_1 = OAc	R_2 = $OCO(CH)_4Pr$	bryostatin 1
R_1 = OH	R_2 = $OCO(CH)_4Pr$	bryostatin 2
R_1 = $OCOBu^i$	R_2 = OCOPr	bryostatin 4
R_1 = $OCOBu^i$	R_2 = OAc	bryostatin 5
R_1 = OCOPr	R_2 = OAc	bryostatin 6
R_1 = OAc	R_2 = OAc	bryostatin 7
R_1 = OCOPr	R_2 = OCOPr	bryostatin 8
R_1 = OCOPr	R_2 = OAc	bryostatin 9
R_1 = $OCOBu^t$	R_2 = H	bryostatin 10
R_1 = OAc	R_2 = H	bryostatin 11
R_1 = OCOPr	R_2 = $OCO(CH)_4Pr$	bryostatin 12
R_1 = OCOPr	R_2 = H	bryostatin 13
R_1 = $OCOBu^t$	R_2 = OH	bryostatin 14
R_1 = OAc	R_2 = $OCO(CH)_4CH(OH)Et$	bryostatin 15



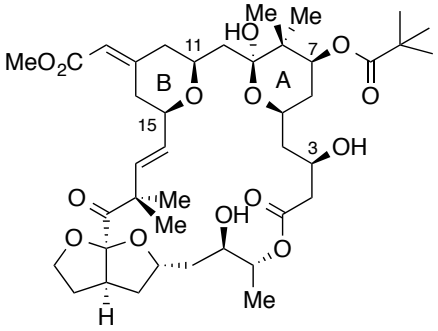
R_1 = OAc	R_2 = $OCO(CH)_4Pr$	bryostatin 3
R_1 = $OCOBu^t$	R_2 = OCOPr	bryostatin 19
R_1 = $OCOBu^t$	R_2 = H	bryostatin 20



X = H	$Y = CO_2Me$	bryostatin 16
$X = CO_2Me$	Y = H	bryostatin 17



bryostatin 18



neristatin 1

Figure 1.1. Molecular structures of bryostatins and structurally related neristatin 1

Biological activities of bryostatins

Initial reports on antineoplastic and cytostatic properties

In 1970, Pettit and coworkers reported that they had observed 168-200% life extension using the extracts from the bryozoan *Bugula neritina* in murine P388 lymphocytic leukemia (PS system) cell lines as developed by the NIH.[2] Later in 1978, the same group reported that bryostatin 1 had shown 52-96% life extension at 10-70 μg/(kg/injection dose) levels and an ED_{50} of 0.89 μg/mL against the murine P388 lymphocytic leukemia (PS system) *in vitro* cell line.[9] In 1983, the authors reported high potency of bryostatin 1 in the form of 34-51% life extension at 37.5-150 μg/kg for L1210 lymphocytic leukemia cell lines and 40-48% life extension at 5-20 μg/kg for M5076 ovarian carcinoma cells and 20-65% curative response in the tumor regression model at 20-40 μg/kg for the same cell line.[4a] Bryostatin 2 was found to show 60% increase in life span at 30 μg/kg for the P388 cell line.[4b] Bryostatin 3 was also found to strongly inhibit growth of P388 cells in the form of 63% life extension at 30 μg/kg.[4a] Bryostatin 4 showed 62% increase in life extension at 46 μg/kg with pronounced cell growth (P388 cell line ED_{50} of 10^{-3}-10^{-4} μg/mL).[4c] Similarly various other members of the bryostatin family also showed antineoplastic properties, as tabulated in Table 1.[10] Researchers from the Johns Hopkins Oncology Center found that maximum inhibition of leukemic growth in acute nonlymphocytic leukemia (ANLL) cells occurred at concentrations as low as 10^{-9} to 10^{-7} mol/L bryostatin 1.[11] Bryostatin 1 was also shown to have some growth inhibitory effect on the MCF-7 human breast cancer cells at relatively higher concentration of 100 nM.[12] DNA synthesis in the human lung cancer cell line A549 suffered 75% inhibition at 10 nM of bryostatin 1.[13] Eventually, preclinical data on the antitumor activities of bryostatin 1

Table 1.1. ED_{50} values and life span extension data with various bryostatins

Compounds	ED_{50} (PS leukemia)	Life span extension[a]
Bryostatin 1	10^{-4} μg/mL	52-96% at 10-70 μg/kg (P388) 34-51% at 37.5-150 μg/kg (L1210) 31-68% at 5-40 μg/kg (M531)
Bryostatin 2	10^{-4} μg/mL	60% at 30 μg/kg (P388)
Bryostatin 3		63% at 30 μg/kg (P388)
Bryostatin 4	10^{-3}-10^{-4} μg/mL	62% at 46 μg/kg (P388)
Bryostatin 5	1.3×10^{-3}-2.6×10^{-4} μg/mL	88% at 185 μg/kg (P388)
Bryostatin 6	1.0×10^{-5} μg/mL	82% at 185 μg/kg (P388)
Bryostatin 7	2.6×10^{-5} μg/mL	77% at 92 μg/kg (P388)
Bryostatin 8		74% at 110 μg/kg (P388)
Bryostatin 9	1.2×10^{-3} μg/mL	40% at 80 μg/kg (P388)
Bryostatin 10	2.6×10^{-4} μg/mL	34% at 10 μg/kg (P388)
Bryostatin 11	1.8×10^{-5} μg/mL	64% at 92.5 μg/kg (P388)
Bryostatin 12	0.014 μg/mL	47-68% at 30-50 μg/kg (P388)
Bryostatin 13	0.0054 μg/mL	

[a]The unit corresponds to injection/dose.

against solid tumor cell lines, including M5076 reticulum cell sarcoma and B-16 melanoma, stimulated its development in the treatment of human cancers.[14] Additionally, bryostatin 1 was also found to reverse multidrug resistance in various cancer cell lines.[15]

Effects on hematopoiesis

Bryostatin 1 was found to stimulate blast forming unit-erythrocyte (BFU-E) granulocyte-macrophage colony forming unit (CFU-GM) in a concentration-dependent fashion, especially in the presence of interleukin 6 (IL-6) or granulocyte colony-stimulating factor (G-CSF).[16] Bryostatin 1 was also found to modulate the proliferation and lineage commitment of CD34+ cells in the presence of interleukin 3 (IL-3) or granulocyte-macrophage colony-stimulating factor (GM-CSF), resulting in an increase in macrophage/neutrophil colonies and a decrease in eosinophilic ones.[17] However, single cell cultures of CD34+ cells were not stimulated by bryostatin 1 alone and later it was postulated that accessory cells such as T cells, stimulated by bryostatin 1, produce GM-CSF and/or IL-3 and mediates the process.[18] In another study with a bryostatin 1 sample supplied by Dr. G. R. Pettit, it was revealed that bryostatin 1 could, mediated by the secretion of tumor necrosis factor α (TNFα) by CD14+ cells, inhibit unpurified bone marrow progenitor cell colony formation.[19]

Effects on immune functions

Initial studies with bryostatins revealed that they could increase cytotoxic T-cell proliferation and function via enhancing the functions of interleukins IL-2 and IL-4. IL-2 receptor expression on CD4+ and CD8+ cells was triggered by bryostatin 1.[20]

Additionally, when lymphocytes obtained from tumor-draining lymph nodes in MCA-105 tumor-bearing mice were incubated with the combination of bryostatin 1 and a calcium ionophore, followed by IL-2, these cells exhibited significant expansion *in vitro* and showed antitumor activity when injected back into the tumor-bearing mice.[21] An *in vitro* linear relationship between the concentration of bryostatin 1 and the reduction of time to platelet activation, maximal rate of aggregation, and stimulated platelet ATP release was also observed.[22] Bryostatin 1 was also found to activate monocytes *in vitro*.[23] Recent studies of bryostatin 1 have shown dual actions of bryostatin 1: inhibiting acute HIV-1 infection as well as reactivating latent HIV, without bystander T-cell activation or cytotoxicity.[24] These results bring bryostatin 1 to the long queue of promising leads against HIV along with highly active antiretroviral treatment (HAART).

Effects on cellular differentiation

Cellular differentiation of human promyelocytic leukemia cells (HL-60) is associated with a reduction in proliferation. Interestingly, bryostatin 1 was found to induce monocytic differentiation of HL-60 cells at nanomolar concentrations.[25] Bryostatin 1 was also found to stimulate differentiation in other promyelocytic leukemia cell lines, NB4 and acute myeloid leukemia (AML) cells, such as the myelomonocytic cell line U937.[26]

Synergistic effects with other anticancer agents

Although bryostatin 1 showed promising antitumor activities as a single agent, the most exciting *in vitro* findings are related to its ability to sensitize to other anticancer

agents. Pre-incubation of AML blast cells with bryostatin 1 increases arabinofuranosyl cytidine (Ara-C)-induced apoptosis, which is likely the result of increased intracellular accumulation of a toxic metabolite 1-β-D-arabinofuranosylcytosine 5'-triphosphate (Ara-CTP) (Table 1.2).[27] WSU-CLL (now considered as pre-B cell leukemia REH cells[28]) severe combined immunodeficiency (SCID) mouse xenografts had an increase in tumor cell apoptosis using 2-chlorodeoxyadenosine (2-CDA) after pretreatment with bryostatin 1.[29] Bryostatin 1 enhanced the cytotoxic effects of taxol on U937 human leukemia cells, cisplatin on cervical carcinoma HeLa cells, and vincristine on WSU-DLCL2 B-cell non-Hodgkin lymphoma cells.[30] The combination of bryostatin 1 and vincristine eliminated tumor completely in 2 of 5 SCID mouse Waldenstrom's macroglobulinaemia xenografts model while either agent alone could not cure any tumor.[31] Additionally, bryostatin 1 synergistically inhibited growth of murine leukemia P388 and showed synergism with

Table 1.2. Synergism of bryostatin 1 with other anticancer agents *in vitro*[32]

Tumor type (cell line)	Anticancer agents
AML	Cytarabine (Ara-C)
CLL (WSU-CLL)[a]	2-chlorodeoxyadenosine (2-CDA)
Leukemia (U937)	Paclitaxel
NHL (WSU-DLCL2)	Vincristine
Cervix (HeLa)	Cisplatin
Waldenstrom's	Vincristine
Breast	Tamoxifen

[a]now considered as pre-B cell leukemia REH cells[28]

auristatin PE and dolastatin 10 in WSU-CLL (now considered as pre-B cell leukemia REH cells[28]) cells.[33] While bryostatin 1 showed some protective effects against radiation on CFU-GM cells, it was also shown to sensitize HL-60 cells to radiation without DNA fragmentation or increase in apoptosis.[34]

Activities on central nervous system (CNS) functions

Daniel Alkon's laboratory at the Blanchette Rockefeller Neurosciences Institute recently found that bryostatin 1 enhanced learning and memory, and showed antidepressant activity in *Hermissenda* and rat models.[35] Bryostatin 1 was found to activate α-secretase at subnanomolar concentrations, thereby enhancing the secretion of α-secretase product soluble amyloid precursor protein $(sAPP)_{\alpha}$ in fibroblasts from Alzheimer's disease (AD) patients, and to reduce brain $A\beta_{40}$ and $A\beta_{42}$ accumulation in AD double-transgenic mice.[36] Additionally, bryostatin 1 showed neuroprotective activity by rescuing ischemia-induced deficits in synaptogenesis.[37]

Clinical trials with bryostatin 1

Promising initial reports on bryostatin 1 inspired the entry of the marine natural product to clinical trials. Phase 1 studies of bryostatin 1 used as a single agent were not very encouraging and showed many side effects such as myalgia, nausea, anorexia, etc.[38] Interestingly, myelosuppression, a common toxicity associated with many conventional cytotoxic drugs, is not a side effect of bryostatin 1. This inspired bryostatin's use in combination with other anticancer agents. Phase 1 trials in combination with paclitaxel

and cisplatin showed mild or no cases of myalgia. Dose-limiting toxicities included cases of tachyarrhythmia, neutropenia, nausea, and anorexia.[39]

These promising results in phase 1 trials inspired phase 2 trials of bryostatin 1 as combined agents in chemotherapy against various cancers. Phase 2 clinical trials of bryostatin 1 in combination with vincristine, AraC, fludarabine, gemcitabine, and temsirolimus gave some promising results along with some inconsistent ones. Myalgia turned out to be a major issue in many cases.[40]

Although continued clinical development of bryostatin 1 as an anticancer agent seems remote at this stage, renewed enthusiasm mounted due to its promising activities against Alzheimer's disease. Recently, the National Health Institute (NIH) has approved a phase 1 clinical trial against AD.[41]

Mechanism of action

The remarkable biological activities of bryostatins and more specifically bryostatin 1 has been attributed to the fact that bryostatin binds to protein kinase C (PKC) with subnanomolar affinity.[42] A brief discussion on PKC follows next.

Protein kinase C (PKC) enzyme

Nishizuka and coworkers first discovered the enzyme protein kinase C (PKC) as a proteolytically activated protein kinase in 1977.[43] PKC is a group of serine/threonine kinases (PKC isozymes) that regulate a variety of cellular processes of signal transduction, including proliferation, apoptosis, differentiation, motility, adhesion, inflammation, etc.[44] There is a substantial amount of evidence linking PKC to

tumorigenesis.[45] However, studying the effects of PKC regulation or misregulation on these processes is impeded by the fact that each isozyme plays distinctive role in these processes in a cell-type-dependent manner.[46] In order to understand various PKC functions, it is important to identify the structural features and the various structural changes that occur during PKC activation.[47]

PKC isozymes can be grouped into three subclasses, namely conventional or classical PKCs (cPKCs), novel PKCs (nPKCs), and atypical PKCs (aPKCs), on the basis of the structural composition of the regulatory domain (Figure 1.2). This composition, in turn, determines cofactor-dependence of the isozymes. The membrane-targeting module

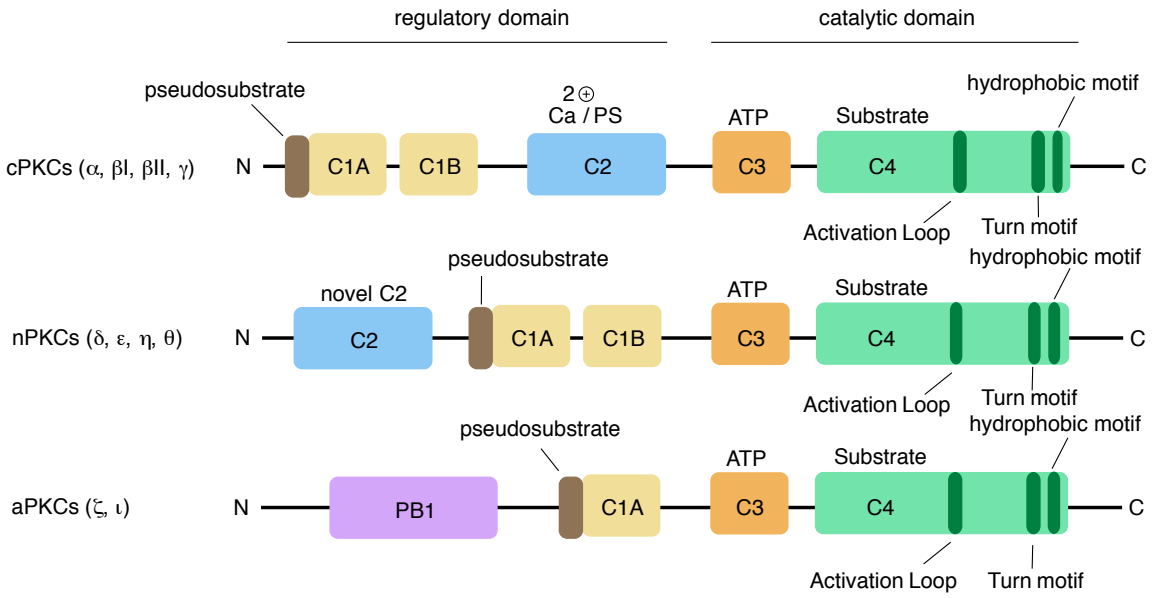


Figure 1.2. Primary structures of cPKCs, nPKCs, and aPKCs.

All the kinases have a conserved kinase core (colored green) and vary in the nature of the regulatory domain. The kinase cores contain activation loops; turn motifs, and hydrophobic motifs. The regulatory domains contain a pseudosubstrate in all kinases. They differ mainly in the composition of membrane targeting modules. (Please see text for detailed description.)

is made of C1 and C2 domains.

The cysteine (Cys)-rich C1 domain is present in all PKC isozymes. It has a binding site for the endogenous ligand, diacylglycerol (DAG). cPKCs and nPKCs have two repetitive C1 domains, but evidence suggests that only one is engaged in ligand binding *in vivo*. For certain isozymes, like PKCδ, it is the C1B domain that binds to DAG or other ligands. For others, such as PKCα, the C1A and C1B domains have equal roles in targeting PKC to membranes. For aPKCs, the C1 domain ligand-binding pocket is impaired and cannot bind to DAG. As the C1 domain is found in many nonkinase enzymes (such as RasGRP1, Munc13-1, etc.), PKCs are not unique in responding to DAG and other C1 domain-binding ligands.[48]

The C2 domain is present in both conventional and novel PKCs. In cPKCs, this domain serves the purpose of a membrane-targeting module that binds anionic phospholipids in a Ca^{2+}-dependent manner. Novel PKCs lack the key residues involved in binding Ca^{2+} and, as a result, they do not bind Ca^{2+} or phosphatidylserine (PS, a phospholipid component). Atypical PKCs do not have the C2 domain; instead, they have a protein-protein interaction PB1 domain. All the PKCs contain a pseudosubstrate sequence that binds to the substrate-binding pocket of PKCs while at rest in an inactive conformation.[47a]

The catalytic domain consists of the kinase core and is highly conserved across all the kinases. The ATP- and substrate-binding site is located in this portion. This portion also contains the activation loop, the turn motif, and the hydrophobic motif.

Various experiments revealed that newly synthesized PKC associates with a membrane compartment in the cell and remains in an open conformation, in which the

autoinhibitory pseudosubstrate is not bound to the substrate-binding cavity.[49] This species of PKC is tethered at the membrane by multiple weak interactions that comprise of weak interaction of the C1 and C2 domain to anionic lipids and interaction of the basic pseudosubstrate with anionic lipids.[50] In this open conformation, the unphosphorylated hydrophobic motif near the C-terminus provides a docking site for 3-phosphoinositide-dependent protein kinase-1 (PDK-1).[51] PDK-1 phosphorylates the activation loop present nearby on the kinase core and is released from the docking site. The activation loop phosphorylates the turn motif and the hydrophobic motif via an intramolecular autophosphorylation mechanism in the case of cPKCs.[52] Phosphorylation of the C-terminal sites results in various conformational changes, thereby locking PKC into a thermally stable conformation. Several reports suggest that phosphorylation of the C-terminus frees the substrate-binding cavity to bind the pseudosubstrate.[53] This forces PKC to adopt a closed conformation, to lose its primary membrane anchor, and localize to the cytosol. This species of PKC is known as the mature species. Various signaling pathways elevate Ca^{2+} levels inside the cell, thereby enabling the C2 domain to bind to Ca^{2+} in cytosolic PKC.[54] Next, diffusion-controlled collisions with the membrane enable the C2 domain to bind to the membrane in a weak interaction. The tethered PKC then finds the membrane-bound ligand DAG and binds to it via a high-affinity interaction with the C1 domain.[55] The energy released in the process is used to undock the pseudosubstrate from the substrate-binding cavity. In this open conformation, mature PKC binds substrates and effects postphosphorylation downstream signaling. As the nPKCs do not bind to Ca^{2+}, their rate of translocation to the membrane is an order lower than the cPKCs and depends on the decreased probability of finding DAG. Although

PKCs at this stage are highly sensitive towards dephosphorylation, molecular chaperone Hsp70 enables the rephosphorylation of PKCs.[56] In the absence of such a process, dephosphorylated PKC localizes to the detergent-insoluble fraction of cells, where it is eventually proteolysed via various pathways (Figure 1.3).

PKC as potential therapeutic target

Since their discovery more than 35 years ago, an enormous amount of research work implicated PKC in almost every cellular process of signal transduction.[45] PKC isoforms are now believed to have both positive and negative influences on proliferation. PKCα, the most studied PKC isoform, can be antiproliferative as well as pro-proliferative.[57] PKCδ was found to influence cell cycle progression by preventing cells from entering S-phase and M-phase. However, it also activated the MAPK pathway, thereby having proliferative effects.[45] PKCβ and PKCε were found to play crucial roles in differentiation.[58] PKC can regulate the transport of integrins to and from the plasma membrane and the presence of integrins at the cell surface is key to proper cell adhesion. Both PKCα and PKCε associate with integrins.[59] PKC isoforms can also phosphorylate myristoylated alanine-rich C-kinase substrate (MARCKS). Consequently, this protein loses its membrane-binding ability and dissociates from the plasma membrane. This can, in turn, facilitate dissociation of the actin cytoskeleton from plasma membrane.[60] Several PKC isoforms (PKCδ, ε, θ, and ζ) are substrates for the enzymes that execute programed cell death, known as caspases. Caspases cleave PKCs in the hinge region and release a constitutively active catalytic domain, which has pro-apoptotic activity.[61] Formation of β-amyloid (Aβ) plaques is considered as a pathological signature of Alzheimer's disease.[62]

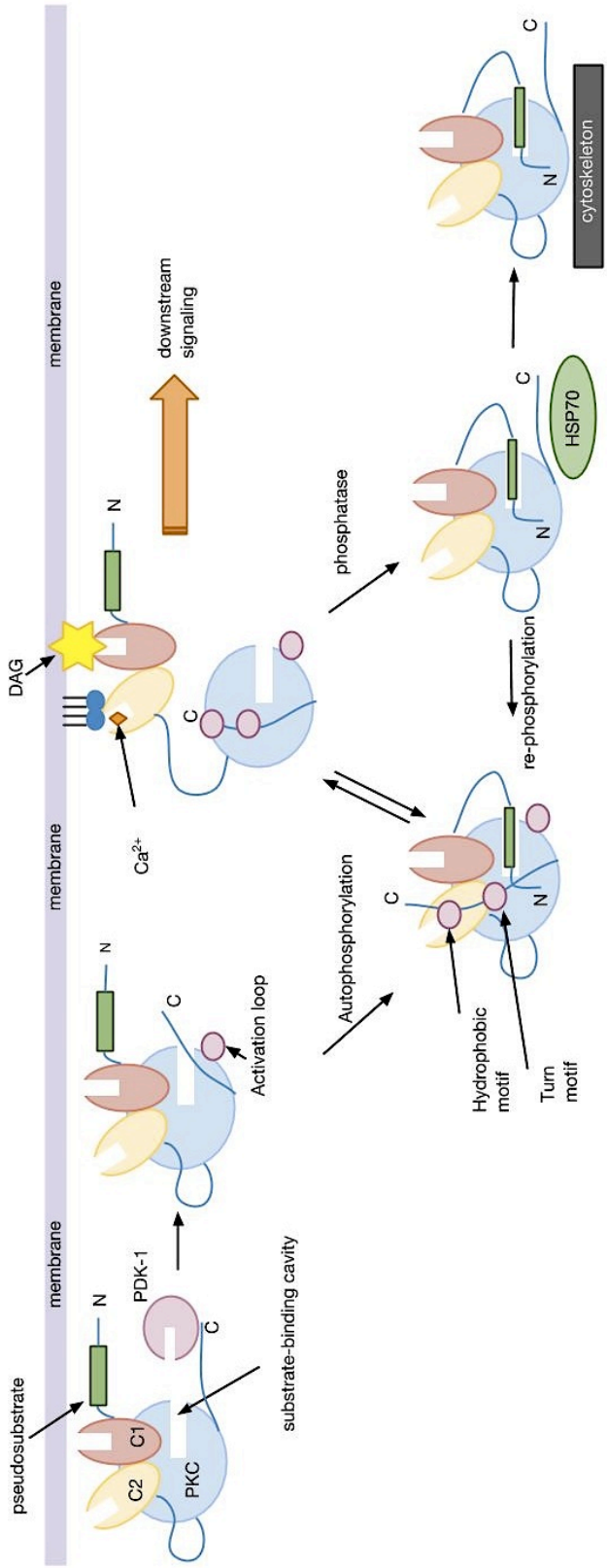


Figure 1.3. Activation pathway of PKC

The amyloidogenic Aβ fragment results from β-secretase-mediated cleavage of amyloid precursor protein (APP) to generate an NH_2 terminus and a further cleavage by γ-secretase to generate Aβ peptide that can oligomerize to generate plaques. On the other hand, α-secretase-mediated cleavage of APP at a different site generates soluble APP (sAPP), which is considered to be nontoxic. PKC isozymes α, ε, and possibly other isozymes can activate the α-secretase-mediated cleavage of sAPP directly or indirectly through phosphorylation of extracellular-signal-regulated kinase (ERK1/2). Evidence suggests that sAPP production by α-secretase competitively reduces Aβ production by the β- and γ-secretases.[63] Although PKC is involved in many cellular functions, the mechanisms of actions depend on specific context, isoform types, and cell types. To understand the mechanisms in greater detail, it is important to find out the various factors that determine PKC actions.

Phorbol esters as potent PKC modulators

Various natural products can be used as tools for identifying molecular targets and this information can lead to the development of various lead compounds for fighting human ailments. The phorbol esters are one of the prime examples of this idea, especially regarding PKC (Figure 1.4). Phorbol esters were isolated and identified from croton oil, the seed oil from *Croton tiglium*.[64] The croton oil was known as an irritant and later found to promote tumor formation in the two-stage model of mouse skin carcinogenesis.[65] Phorbol 12-myristate 13-acetate (PMA) was found to be the most potent of all the compounds isolated but was too lipophilic for target identification. A designed analogue ^{3}H-labeled phorbol 12, 13-dibutyrate ([^{3}H]PDBu) permitted the demonstration

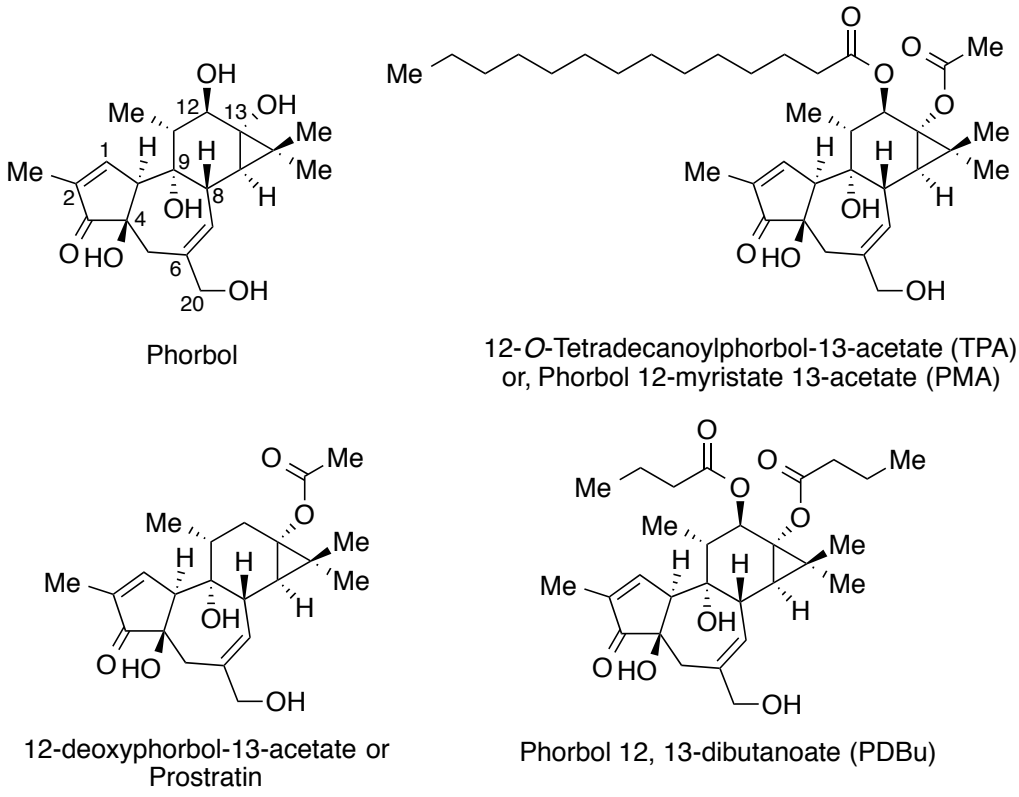


Figure 1.4. Molecular structures of various phorbol related compounds

and characterization of a specific receptor for the phorbol esters, thanks to the Blumberg group.[66] Later in a hallmark work by Nishizuka, et al., it was established that PKC was indeed the molecular target for phorbol esters.[67] Around the same time, in a further enlightening work by Kraft, *et al*., translocation of PKC by phorbol esters was found to depend on lipophilicity of those esters.[68] All these results and the research that followed since then have firmly put phorbol esters into a pharmacological context. However, as mentioned earlier, PKC is not the sole target for DAG/ phorbol esters and at least 20 different C1 domain-containing transducing proteins bind with DAG/ phorbol esters and result in an extensive network of downstream signaling. The outstanding questions that pose considerable challenges are related to specificity and selectivity of various signaling

pathways chosen by cells. Are there any ways for the small molecules (DAG, phorbol esters) to differentiate between various target proteins and their signaling pathways?

A step towards answering these questions was provided by Hecker and coworkers, who demonstrated that different phorbol derivatives could induce different patterns of biological response. This indicated that it would be possible to structurally manipulate the ligands to affect subpathways of response.[69] It was further shown by Blumberg, et al. that prostratin, although a phorbol ester, is not a tumor-promoter and inhibited PMA-induced tumor promotion in CD-1 mouse skin.[70]

Differential effects of bryostatin 1 and phorbol esters

Bryostatin 1 was initially shown to be a potent PKC ligand and had similar effects to those of the phorbol esters.[42, 71] Later, it was found that in some instances, bryostatin 1 failed to induce a typical phorbol ester-like response and more importantly blocked phorbol ester-induced differentiation of human promyelocytic leukemia cells HL-60.[72] This antagonism was found to be the general trend for bryostatin 1 rather than an exception. Bryostatin 1 lifted the phorbol ester-induced blockage of differentiation in hexamethylene bisacetamide-treated Friend erythroleukemia cells.[73] Bryostatin 1 was also found to restore cell-cell communication that was blocked by phorbol esters.[74] In a very important observation, Blumberg's laboratory found that bryostatin 1 failed to function as a tumor promoter in mouse skin and indeed inhibited tumor promotion by phorbol ester.[75]

Another aspect of mechanistic difference between bryostatin 1 and PMA was observed in studies on the downregulation of PKC isoforms. PMA and bryostatin 1 both

caused dose-dependent downregulation of PKCα but for PKCδ, PMA and bryostatin 1 had differential effects. PMA downregulated PKCδ but bryostatin 1 afforded a biphasic pattern, with maximal downregulation at low doses and protection of PKCδ from downregulation at higher doses.[76] This effect appeared to have a direct correlation with the observation from Blumberg laboratory that suppression of PKCδ expression with siRNA rendered the proliferative response insensitive to bryostatin 1 and overexpression of PKCδ inhibited cell growth in HOP92 cells. These results are consistent with the usual antiproliferative effect of PKCδ.[77]

Mechanistic differences between bryostatin 1 and PMA were also observed in the studies of translocation patterns of PKCδ. PMA induces translocation initially to the plasma membrane and subsequently to the internal membranes and the nuclear membrane. Bryostatin 1, on the other hand, induces translocation directly to these internal membranes, with little or no translocation to the plasma membrane.[78]

Nature of interactions between ligands and C1 domain

Various NMR studies on the structure of DAG-responsive C1 domains along with a crystal structure of phorbol ester bound to C1B domain of PKCδ have revealed a detailed picture of how the C1 domain functions as a hydrophobic switch.[79] The phorbol ester binds to the hydrophilic cleft formed by the pulled apart strands of a β-sheet at the top of the C1B domain. The surface surrounding this cleft is hydrophobic and phorbol ester, upon binding, completes the hydrophobic surface. This, in turn, favors binding of the C1 domain – phorbol ester complex into the lipid membrane. The C1 domain does not change conformation significantly upon phorbol ester binding and this rules out any

allosteric regulation for its mode of action. Instead, the binding changes the association preference of the hydrophobic face of the C1 domain. For various ligands, the coverage of the hydrophilic cleft of the C1 domain could be similar (discussed more later). However, the diverse forms of hydrophobic side chains projecting from the ligand can be of special significance. As mentioned earlier, different acyl substituents on the phorbol ester can lead to very different biological responses (prostratin versus PMA). As mentioned earlier, the C1 domain binds to the plasma membrane and then finds its ligand DAG. However, this binding is an equilibrium process and the association with the ligand stabilizes the binding, making the rate of dissociation slower and shifting the equilibrium towards binding to the membrane. This process, expectedly, can be regulated by the ligands like phorbol esters. For relatively hydrophilic ligands, their increased presence in the cytosol may implicate a prior binding to the C1 domain.[80]

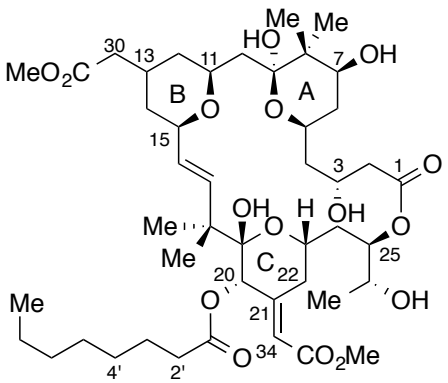
Additionally, the actual binding of ligand with the C1 domain is measured in the presence of phospholipid, which forms the ternary complex of ligand – C1 domain – lipid. Therefore, the interaction with lipid also needs to be considered while investigating the biological profile of the ligands.

Finally, an interesting observation made by the Blumberg group needs to be mentioned at this point. Tumor promoters like PMA and the indole alkaloids like indolactam and octylindolactam were selectively dependent on the C1B domain of PKCδ in mouse 3T3 cells, whereas compounds that were not tumor promoters such as prostratin, mezerein, or 12-deoxyphorbol 13-phenylacetate, bryostatin 1 bound to both C1A and C1B domains.[81]

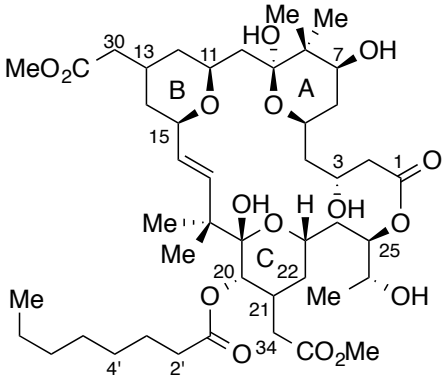
Proposed pharmacophoric elements of bryostatins

Early structure and activity relationship studies on bryostatin were achieved through a collaborative effort between the Pettit, Blumberg, and Wender groups.[82] Comparison of the binding affinities of bryostatins 1-10, 16, 17, and 18 gave insight into the structural features responsible for binding. Varying ester functional groups on the C7 and the C20 did not change the binding affinities significantly (Figure 1.5). Similarly, epoxidation of the C13-C30 olefin of bryostatin 4 did not reduce the binding affinity; neither did the reduction of the C13-C30 bond and the unsaturated side chain at C20 of bryostatin 2.[82a] In contrast, bryostatin 2 derivatives in which both the C13-C30 and C21-C34 olefinic bonds are hydrogenated showed marked reduction in binding affinities (Figure 1.5).[4g, 82a] Elimination of the C19 hydroxyl group as found in bryostatin 16 and 17 eliminates significant binding ability. Inversion of stereochemistry at C26 also reduced the binding affinity to some extent. Finally, acetylation of the C26 hydroxyl group reduced the binding affinity dramatically.[82b] Wender concluded from these observations that C4-C16 domains of bryostatins are less significant than the corresponding C19-C26 domains in terms of binding.

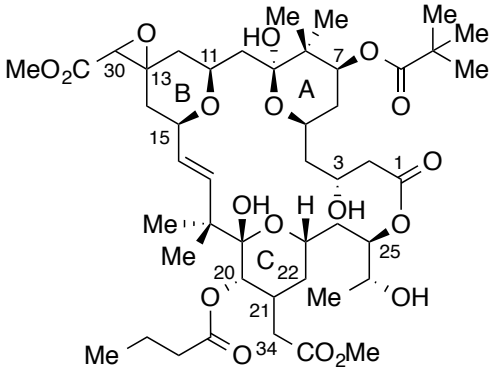
These observations were combined with an already developed computational and structure-activity relationship study related to phorbol compounds and DAG analogues.[83] Structure-activity and computational studies of phorbol derivatives indicated that the long-chain esters on C12 or C13 hydroxyl groups and hydroxyl groups at C4, C9, and C20 are necessary for binding. The spatial coordinates of the heteroatoms in phorbol conformers and (*S*)-1,2-diacyl-*sn*-glycerol conformers showed good correlation. For bryostatin 1, the heteroatoms that were found to have similar or better correlations are



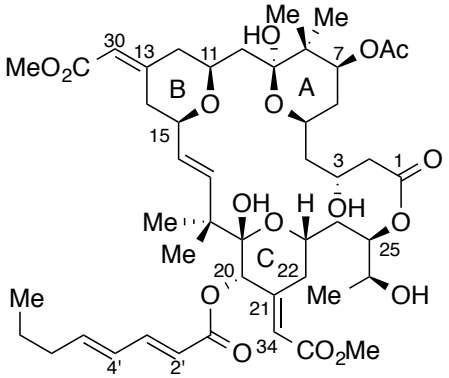
(13, 30, 2', 3', 4', 5'-hexahydro)-bryostatin 2
K_i = 9.61 ± 0.94 nM



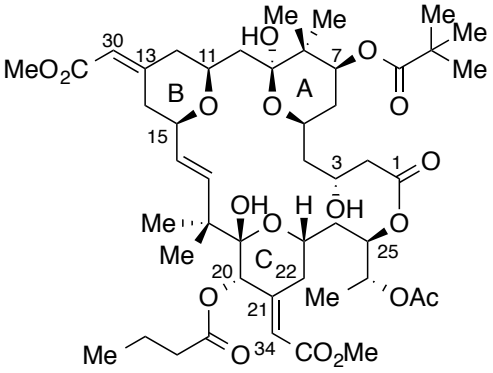
(13, 30, 21, 34, 2', 3', 4', 5'-octahydro)-bryostatin 2
K_i = 473 ± 96 nM



(13, 30-epoxy)-bryostatin 4
K_i = 0.54 ± 0.07 nM



(26-epi)-bryostatin 1
K_i = 32.6 ± 0.6 nM



(26-acetoxy)-bryostatin 4
K_i >> 100 nM

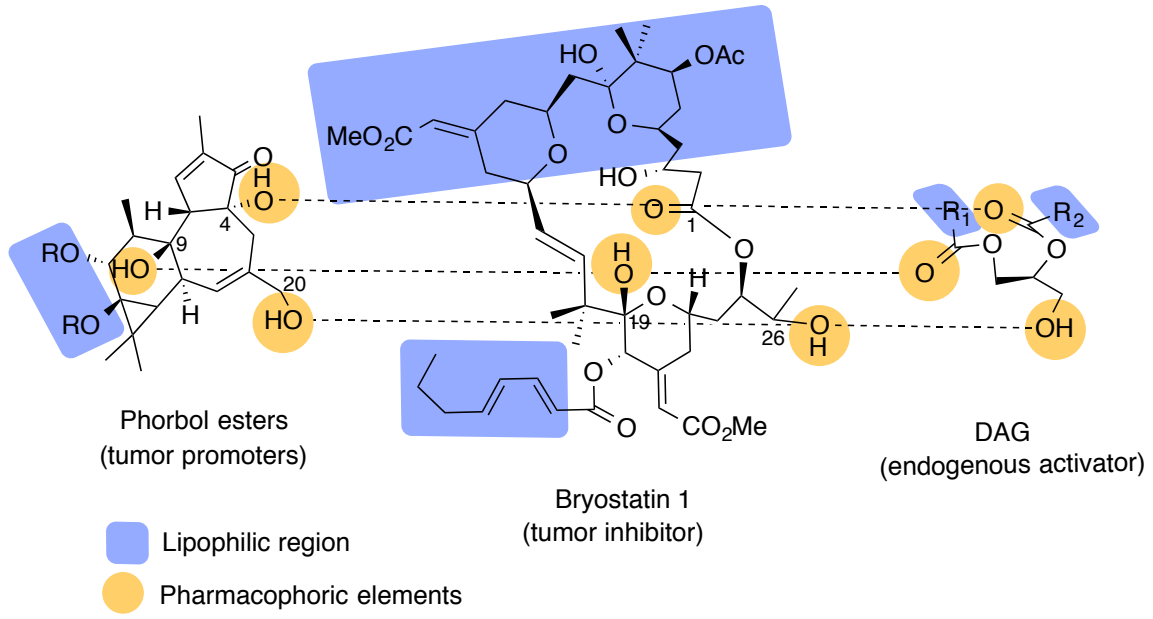
bryostatin 1	K_i = 1.35 ± 0.17 nM
bryostatin 2	K_i = 5.86 ± 1.13 nM
bryostatin 3	K_i = 2.75 ± 0.05 nM
bryostatin 4	K_i = 1.30 ± 0.19 nM
bryostatin 5	K_i = 1.04 ± 0.10 nM
bryostatin 6	K_i = 1.18 ± 0.29 nM
bryostatin 7	K_i = 0.84 ± 0.07 nM
bryostatin 8	K_i = 1.72 ± 0.10 nM
bryostatin 9	K_i = 1.31 ± 0.00 nM
bryostatin 10	K_i = 3.36 ± 0.06 nM
bryostatin 16	K_i = 118 ± 2 nM
bryostatin 17	K_i = 188 ± 7 nM
bryostatin 18	K_i = 4.82 ± 0.06 nM

Figure 1.5. Binding affinities (K_i, nM) to mixture of PKC isoforms of bryostatins and various derivatives

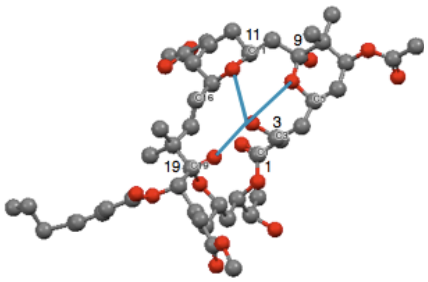
C19, C26 hydroxyl groups, and C1 carbonyl oxygen (Figure 1.6a). Although bryostatin 1 does not have hydrophobic esters in similar position to that of phorbol esters, the largely lipophilic body of the molecule can be expected to occupy the top surface of the hydrophilic cleft in the C1 domain.[82b] Although this above hypothesis conforms to most of the observations, it does not necessarily correlate with the reality. In 1995, when a crystal structure of phorbol 13-acetate bound to C1B domain of PKCδ was published, it was found that 9-OH in the phorbol ester has no interaction with the C1 domain even though it was assumed to be one of the pharmacophore.[79c] This striking inconsistency could be due to the lipid-ligand interaction near the membrane.[80] Nonetheless, Wender went on to propose that the northern hemisphere of bryostatin 1 (A- and B-rings) serves as a spacer domain and rigidifies the orientation for the pharmacophores (C1, C19, and C26) while binding to the C1 domain.[84]

A series of analogues prepared by Wender and coworkers were used to study the role of the C3 hydroxyl group. From solution NMR studies and crystallographic data, the C3 hydroxyl group is known to participate in an intramolecular hydrogen-bonding network with the C19-OH and the oxygen atoms of the A- and B-rings. NMR solution structure analysis and computational studies for these analogues revealed that the C3-epi analogue and the C3-deoxy analogue both had very different conformations compared to bryostatin 1 and C3 failed to form a hydrogen-bonding network with the C19-OH and the A- and B-ring pyran oxygen atoms. Although direct binding to the protein was not ruled out, the diminished binding affinity (285 nM and 297 nM versus 3.4 nM, Figure 1.6c) was considered to be the result of the absence of the transannular hydrogen-bonding network present in bryostatin 1.[85]

(a)



(b)



(c)

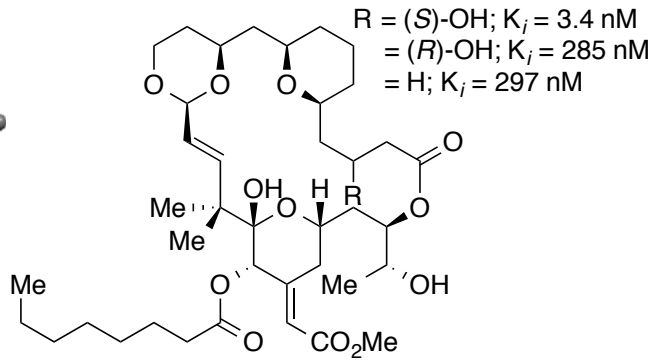


Figure 1.6. Pharmacophoric comparison of the PKC activators phorbol, bryostatin, and DAG, hydrogen bonding interactions, and role of C3 hydroxyl moiety

(a) Computation studies showed that the spatial coordinates of the three heteroatoms correlated with each other very closely. (b) The crystal structure of bryostatin 1 (BOKKIV) shows transannular hydrogen-bonding interactions (in blue lines) between C19 hemiketal hydrogen and C3 hydroxyl oxygen, C3 hydroxyl hydrogen and A- and B-pyran ring oxygen atoms. (c) Structures of analogues for studying the role of C3 hydroxyl group.

Identifying and understanding the mechanisms accountable for heterogeneous PKC responses are highly important not only to predict the actual biological consequences of PKC activation but also to identify useful and selective modulators of the PKC enzyme pathway.

Chemical syntheses of bryostatins

Against the backdrop of the intense engagement of the scientific community in studying the unique biological profiles of bryostatins, it is not surprising to see chemists engaging in finding out efficient ways to prepare molecules of this class in the laboratory. Additionally, in practice, chemical synthesis seems to be the only viable solution for supplying adequate amount of bryostatins for further biological characterization. Isolation of these molecules from their natural sources proved to be a daunting task. For example, some 13000 kg of wet bryozoans were needed to isolate 18 g of bryostatin 1, the most studied member of the family.[86] A continuous effort in this direction seems detrimental to the marine ecology. Aquaculture studies of *Bugula neritina* at a company called CalBioMarine under the supervision of National Cancer Institute failed to sustainably produce bryostatin in desirable amounts. Although Haygood groups' efforts enabled the identification of the gene cluster *bryA* responsible for the production of bryostatin 1 in the marine microbe, expression of the gene was not possible in the laboratory.[87]

Many synthetic research groups have been involved in the total syntheses of bryostatins. Masamune was the first one to synthesize a member of the bryostatin family, bryostatin 7.[88] Later, Evans[89] (bryostatin 2), Yamamura[90] (bryostatin 3), and Trost[91] (bryostatin 16) have demonstrated elegant synthetic routes to access these natural

products. In addition, Hale[92] (formal synthesis of bryostatin 7), Trost[93] (C20-epi-bryostatin 7), and Thomas[94] (20-deoxybryostatin) reported several synthetic routes for the related compounds. Recently, our group reported the first total synthesis of bryostatin 1.[95] Subsequently, Wender and Krische group reported the total syntheses of bryostatin 9 and bryostatin 7, respectively.[96]

Keck's analogue design

Although various total syntheses of bryostatins have been reported since 1990, a more practical strategy towards the preparation of bryostatins is to make analogues, which may retain bryostatins' unique biological profile but shorten the synthetic route to provide relevant quantities of materials for further biological characterization.

Pyran annulation or Keck Yu annulation

Our synthetic strategy towards bryostatin 1 was first realized from the development of the pyran annulation reaction in our group (Figure 1.7).[97] In this process, an aldehyde and a *β*-hydroxyallylsilane can be coupled together in the presence of trimethylsilyl triflate to diastereoselectively produce 2,6-*cis*-disubstituted-4-methylenetetrahydropyran. A similar reaction was also developed independently by Chan-Mo Yu's group and hence, this reaction is now broadly known as the Keck Yu annulation.[96b, 98] Additionally, Marko reported similar reactions based on Sakurai Prins-type cyclization strategy, which he called "the intramolecular silyl-modified Sakurai (ISMS) reaction".[99]

The TMSOTf-promoted annulation of *β*-hydroxyallylsilane **1.3** with the aldehyde

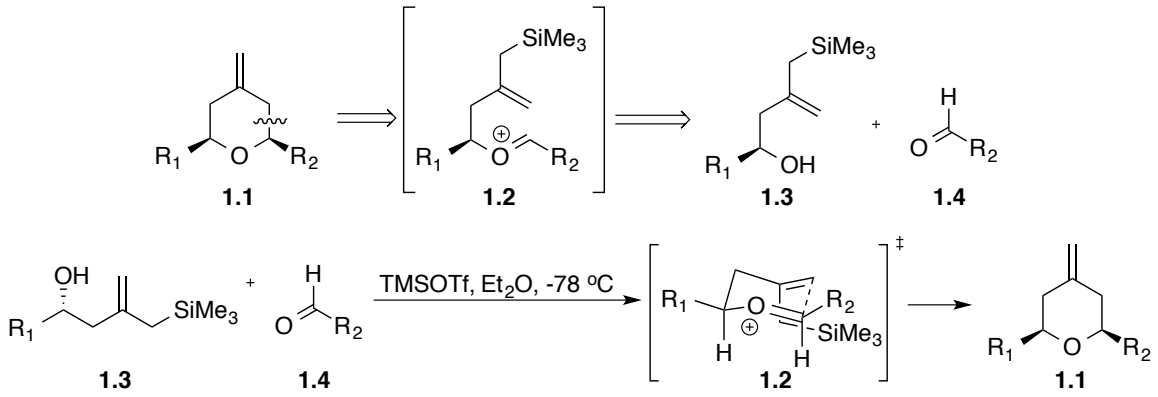


Figure 1.7. Retrosynthetic strategy and transition state of Keck Yu annulation

1.4 occurs rapidly at -78 ^{o}C, possibly via a chair-like transition state **1.2**, which also explains the high diastereoselectivity (Figure 1.7). Et_2O was found to be the best solvent and TMSOTf was the most suitable Lewis acid for this reaction.[100]

Keck's initial analogues of Merle series

Our group utilized the powerful pyran annulation reaction as a tool in the preparation of all the analogues. These analogues were given permanent identifiers as Merle numbers. Figure 1.8 shows the utilization of the key reaction in the preparation of some of the early members of the Merle series, Merle 23 and 28.[101] Additionally, Figure 1.9 depicts several other analogues prepared in our group prior to 2010.[102]

Biological studies of Merle 21-28

The biological studies with the Merle series of compounds have been performed in the laboratory of our collaborator, Dr. Peter Blumberg, at the National Cancer Institute. The goal of the collaboration is to identify the structural features which are required for

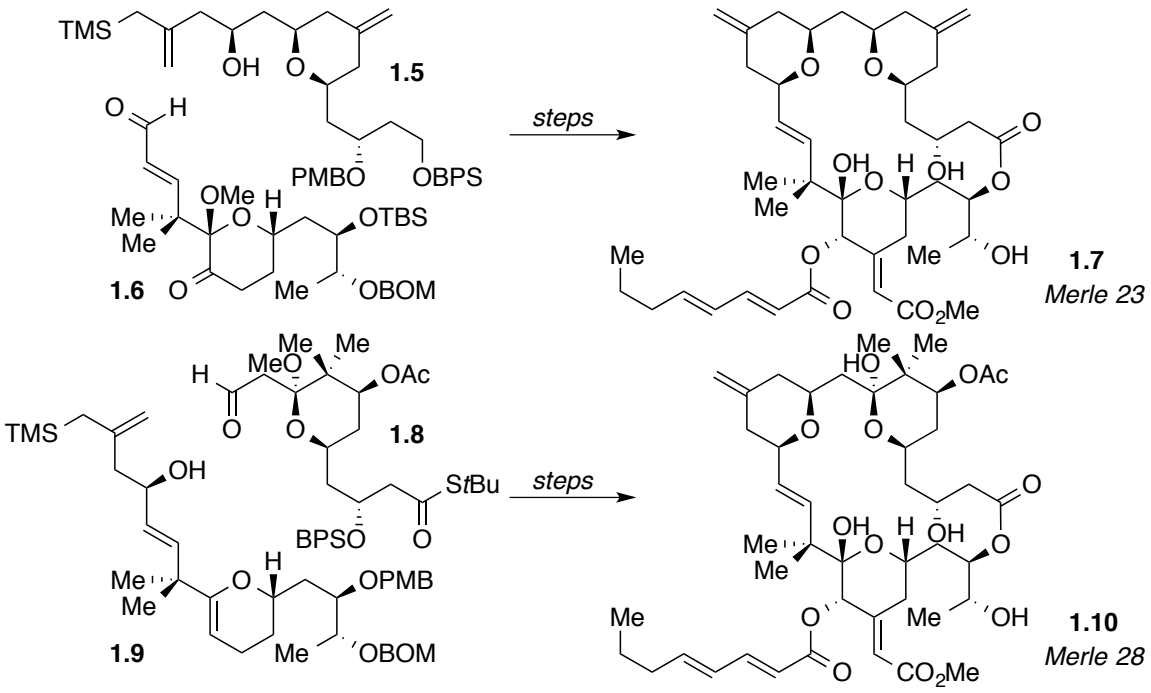
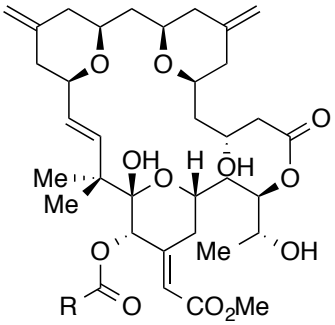
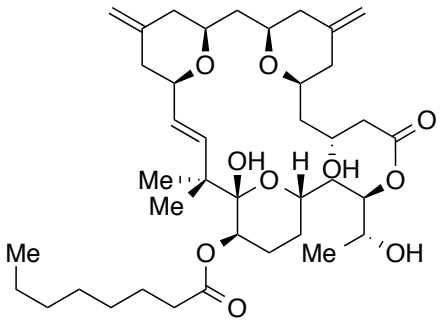


Figure 1.8. Example of some uses of Keck Yu annulation

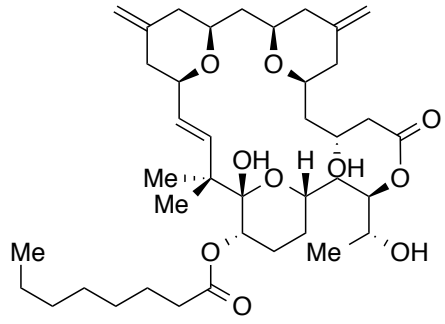


1.11 R = Ph, *Merle 21*

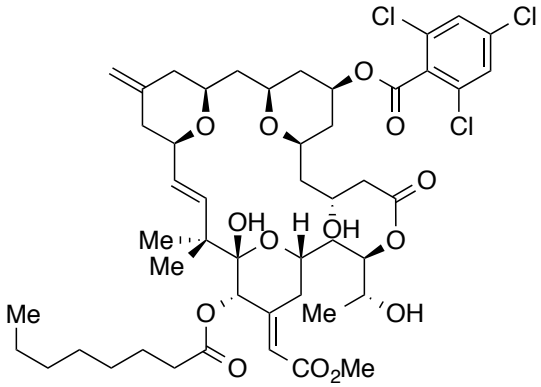
1.12 R = Me , *Merle 22*



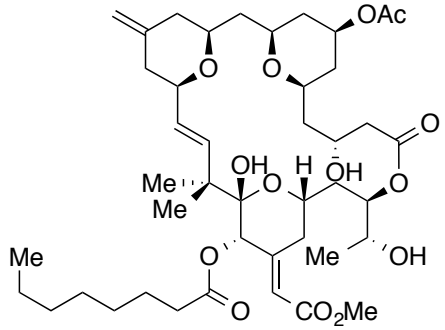
1.13 *Merle 24*



1.14 *Merle 25*



1.15 *Merle 26*



1.16 *Merle 27*

PKCα Binding affinities of analogues

Merle 21, K_i = 0.70 ± 0.01 nM
Merle 22, K_i = 1.05 ± 0.04 nM
Merle 23, K_i = 0.70 ± 0.06 nM
Merle 24, K_i = 37.7 ± 0.5 nM
Merle 25, K_i = 47.1 ± 4.2 nM
Merle 26, K_i = 13.0 ± 3.8 nM
Merle 27, K_i = 3.00 ± 0.6 nM
Merle 28, K_i = 0.52 ± 0.06 nM

Figure 1.9. Structures and binding affinities of Keck analogues of Merle series prepared prior to 2010

both PKC binding and also for biological function as a bryostatin 1 mimic. Among the PKC binding agents, only the bryostatins are known to act as functional antagonists of a subset of PMA-induced biological responses. Conversely, for those responses induced both by the bryostatins and phorbol esters, the greater selectivity of the bryostatins and their weak activity as tumor promoters make them attractive PKC agonists.[103] Therefore, our biological studies began with comparing binding affinities between bryostatin 1, phorbol ester, and the analogues. Additionally, as the high binding affinity of a ligand does not necessarily reflect anything about its biological function, specific cell lines were chosen to examine the subset of responses where bryostatin 1 and phorbol ester show functional antagonism.

Binding affinities of Merle 21-28

The inhibitory dissociation constant (K_i, which indicates binding affinity) of each analogue ligand was determined by the ability of the ligand to displace bound [20-^{3}H]phorbol 12,13-dibutyrate ([^{3}H]PDBu) from mouse recombinant isozyme PKCα in the presence of calcium and phosphatidylserine, using a polyethylene glycol precipitation assay previously described by Blumberg and Lewin.[104] Merle 21, 22, and 23 lacked the polar functional groups in the northern hemisphere of bryostatin 1 along with the C8-*gem*-dimethyl group. However, all of them were found to bind to PKCα with affinities (0.70 nM to 1.05 nM, Figure 1.9) similar to that of bryostatin 1 (0.48 ± 0.03 nM). Additionally, this indicated that subtle changes in the ester functional group at C20 did not change the binding affinity substantially. Merle 24 and 25 both lack the C21 carbomethoxyenoate functional group. While Merle 25 has the right stereochemical

configuration at C20, Merle 24 has the opposite stereochemistry at C20. Interestingly, these analogues showed marked decrease in binding affinity (37 nM & 47 nM), which indicated that the C21 carbomethoxyenoate group plays a role in determining the binding affinity. This also showed that Wender's hypothesis on pharmacophores on bryostatin 1 was not a full description of the way bryostatin binds to PKC. In Merle 26 and 27, the analogues have the C7 acetate and the C7 2,4,6-trichlorobenzoate groups. These analogues also bound to PKCα, but with slightly diminished potency compared to bryostatin 1. Merle 28, on the other hand, retained the functional groups of the bryostatin 1 A-ring and lacked only the C30 carbomethoxy. As expected, Merle 28 bound to PKCα tightly (Figure 1.9).

Investigation of biological functions of Merle 21-28

All these analogues were screened for function by examination of attachment and proliferation of U937 leukemia cells.[105] In this assay, phorbol esters inhibit proliferation and induce attachment. Bryostatin 1 has little effect but blocks the effect of the phorbol ester. Figure 1.10 shows the results with the analogue Merle 23. Merle 21 and 22 showed almost identical results and therefore are not displayed here. Merle 23 showed almost identical behavior in a dose-dependent manner to that of the tumor-promoting phorbol ester PMA and distinctly differed from bryostatin 1.[101a] When used in combination with PMA, bryostatin 1 blocked the responses of the phorbol ester. Merle 23, on the other hand, could not block the responses of PMA. This path-breaking result showed that Wender's hypothesis of the northern hemisphere of bryostatin 1 as a spacer domain is misleading and any drug design based on that would not guarantee a biological response

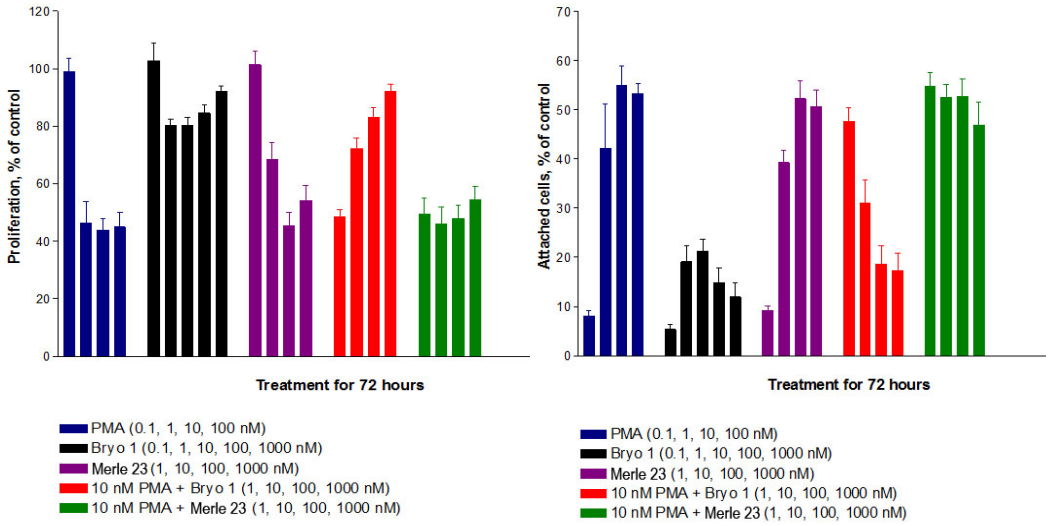


Figure 1.10. Attachment and proliferation assays on U937 leukemia cells

similar to the nontumor-promoter bryostatin 1.

Additionally, Blumberg and coworkers examined Merle 21, 22, and 23 on the human erythroleukemia cell line K562. In these cells, similarly to U937 cells, Merle 23 behaved like PMA for inhibiting cell growth in a dose-dependent manner (Figure 1.11).[102c] However, in contrast to U937 and K562 cells, human prostate cells LNCaP were different. Blumberg observed Merle 23 resembled bryostatin 1 and not PMA in these cells. Merle 23 did not inhibit growth and did not induce apoptosis like bryostatin 1. It also had similar bryostatin-like effect on TNFα secretion and cell cycle analysis studies (Figure 1.12).[106]

Blumberg also reported that the above-mentioned pattern of behavior of Merle 23 relative to PMA and bryostatin 1 depended very much on the specific conditions. It was found that protease inhibitors like lactacystin and MG-132 shifted the response of the LNCaP cells to Merle 23 from bryostatin-like to PMA-like. These findings led to the conclusion that Merle 23 responses relative to PMA and bryostatin 1 depend on the cell

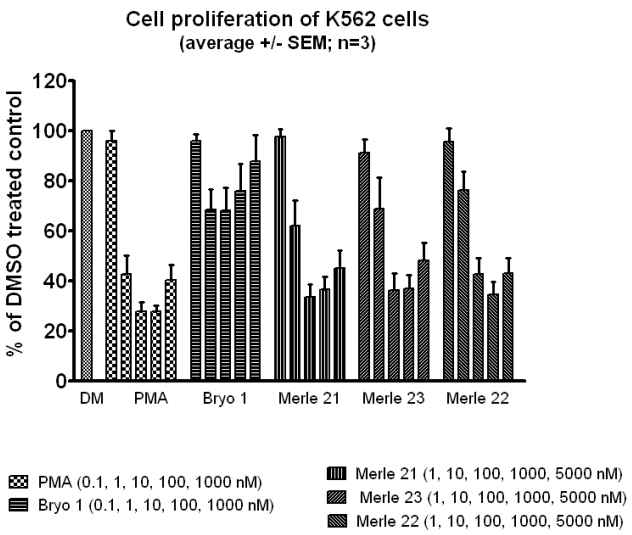


Figure 1.11. Proliferation assay on K562 erythroleukemia cells

type and can be modulated by other agents like lactacystin and MG-132. Blumberg also noticed that for a number of downstream responses in LNCaP cells, Merle 23 showed a duration of response intermediate between those of bryostatin 1 and PMA, with a little more PMA-like character. Additionally, for translocation studies with overexpressed GFP-PKCδ, Merle 23 resembled bryostatin 1, whereas for endogenous PKCδ, Merle 23 was unique and translocated PKCδ to the cytoplasm as well as to the plasma membrane, unlike both phorbol ester and bryostatin.[107] The authors concluded that Merle 23 could not be simply considered to be somewhere in the spectrum of activity between bryostatin 1 and PMA. Instead, Merle 23 should be considered as a unique compound with its own effects.

Merle 26 and 27, which reintroduced C7 acetate and 2,4,6-trichlorobenzoyl groups on Merle 23, still behaved PMA-like on U937 attachment and proliferation assays. These studies along with the diminished potency of these analogues compared to Merle 23 and bryostatin 1 showed that the C7 acetate functional group in bryostatin 1 is

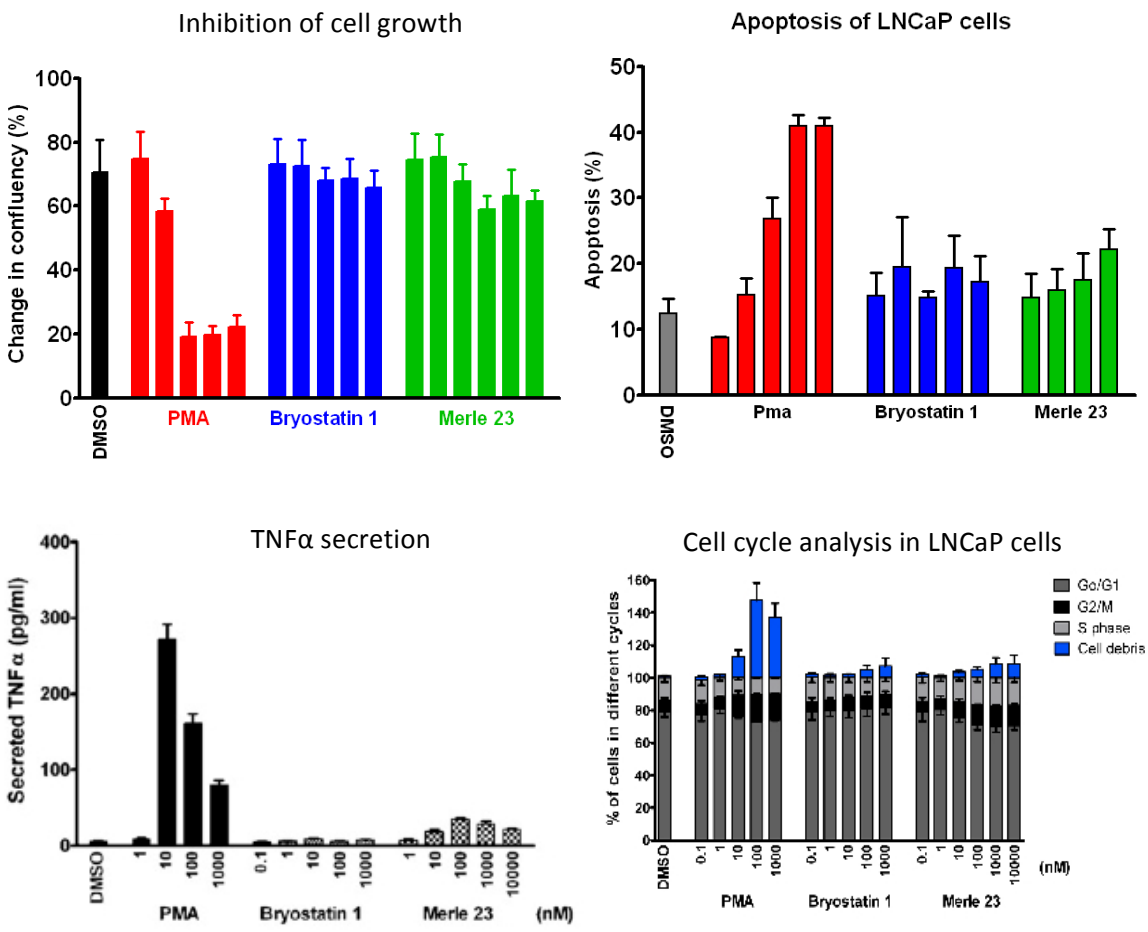


Figure 1.12. Similarity between bryostatin 1 and Merle 23 in LNCaP cells

not the critical determinant for antagonism of PMA-induced biological responses. Complete restoration of polar functional groups on the A-ring as well as C8-*gem*-dimethyl group on Merle 28 shifted the properties towards bryostatin-type responses. While this reinforced the evidence against the hypothesis of A- and B-rings as "spacer domain", it also proved that the C30 carbomethoxy group is not essential and that the A-ring functional groups are critical to obtain bryostatin-like biological responses (Figure 1.13). In a more recent work in collaboration with our group, Blumberg group reported that the origin of the unusual pattern of responses in LNCaP cells compared to U937 cells

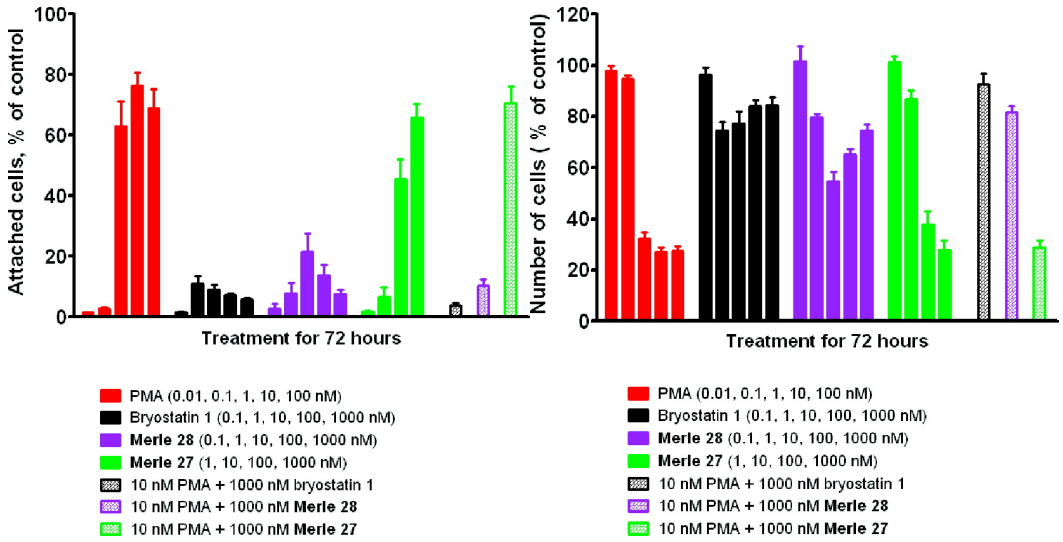


Figure 1.13. Attachment and proliferation assays on U937 cells with Merle 27 and 28

possibly lie downstream of the transcriptional regulation by various Merle compounds.[108]

Synthesis of C9-deoxy bryostatin 1 or Merle 30

So far we had discovered that the C7 acetate and C30 carbomethoxy groups alone do not play the critical roles towards bryostatin-like activities. In the backdrop of these outcomes of our biological studies with the analogues, we decided to further investigate the structure-activity relationships. In line with that, we planned to delete the C9 hydroxy group from the A-ring of bryostatin 1. This C9 hydroxy group is not an ordinary hydroxy functionality. It is in fact a part of the hemiketal group, the other oxygen being part of the A-ring as well. Hemiketals are known to be chemically labile under acidic conditions. Protonation of the hydroxy group can trigger the lone pairs of the other oxygen atom to form an oxocarbenium ion by eliminating a water molecule. This reactive intermediate can then be attacked by nucleophiles such as other water molecules, thiols, or alcohols.

We hypothesized that a similar event in the surrounding cellular environment could perhaps be responsible for bryostatin's special activities.

Computational studies of Merle 30 by Megan L. Peach

Etai and coworkers carried out a computational study of bryostatin 1-bound PKC where they found four intermolecular hydrogen bonds. In this model, bryostatin fits well into the same cavity where phorbol ester was found to bind in a crystal structure. The four hydrogen bonds are found to form with the C26-OH to both the NH of Thr242 and the carbonyl of Leu251, the C35 carbonyl to the NH of Gly253, and the C9-OH to the carbonyl of Met239.[109]

Our collaborator, Dr. Megan Peach, performed a conformational search of bryostatin 1 in implicit water and octanol solvents. The global energy-minimum conformation found in both solvents matched with the crystal[5] and NMR[110] conformations. The four lowest energy conformations are characterized by their intramolecular hydrogen-bonding network between the C3-OH group and the pyran oxygen atoms of the A- and B-rings, and another hydrogen-bonding between the proton of the C9-OH and the oxygen atom of the C3-OH group. Corresponding C9-deoxy bryostatin conformations were then built by simply replacing the C9-OH with a hydrogen atom. These conformations of bryostatin 1 and Merle 30 were docked into the crystal structure of the C1B domain of PKCδ (Figure 1.14). The docking studies revealed that bryostatin 1 or Merle 30 did not undergo any conformational change upon binding to the C1 domain. In both the compounds, the C26 OH formed similar hydrogen-binding interactions with the Leu251 carbonyl and Thr242 NH groups. The difference with Itai's

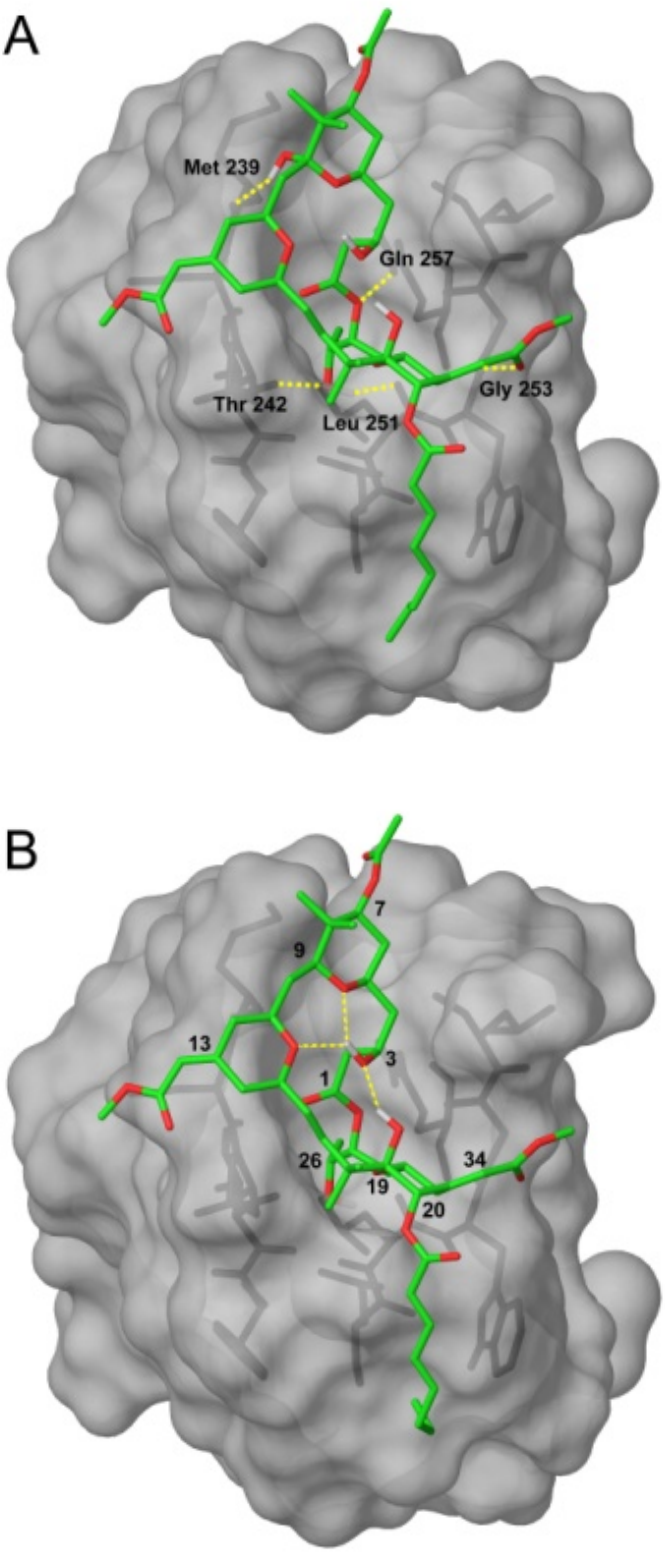


Figure 1.14. Bryostatin 1 (A) and Merle 30 (B) docked into the C1B domain of PKCδ

observation was in the fact that the ester oxygen atom adjacent to C1 appeared to make weak hydrogen-bonding interaction with NH of Gln 257. The methoxy carbonyl group at C34 extends over the edge of the binding site and hydrogen bonds to the backbone Gly253 NH. Bryostatin also forms a hydrogen-bonding with C9 OH to Met239, which Merle 30 cannot form. This hydrogen-bonding is also absent in phorbol esters. These results from the computational studies supported our hypothesis that a C9-OH could be essential for bryostatin-like activity.

Retrosynthesis of Merle 30

The retrosynthetic analysis of the C9-deoxy bryostatin 1 (Merle 30) is outlined in Figure 1.15. We envisaged that Merle 30 could arise from late stage deletion of the C9 hydroxy group in the ketone intermediate **1.18**. The macrolactone was disconnected at C1 ester bond keeping a Yamaguchi macrolactonization reaction in mind. The unsaturated ester functionalities on C13, C20, and C21 would also be installed late onto the macrolactone core of Merle 30. The B-ring pyran of the ketone **1.18** was disconnected at C11-C12 and C11-O (B-ring pyran oxygen) bonds complying with our pyran annulation strategy to yield synthetic equivalents in the form of the C-ring β-hydroxyallyl silane **1.19** and the A-ring aldehyde **1.20**. The highly convergent union of these two equally complex intermediates with four stereocenters each would not only construct the B-ring pyran but would also install the desired stereocenter at C11. The A-ring aldehyde **1.20** could be obtained from the open chain intermediate **1.21** through a cleavage of the olefin at C9 followed by a ketalization (Figure 1.16). We envisioned that the installation of the C8 geminal dimethyl group could be achieved via a chelation controlled addition of the

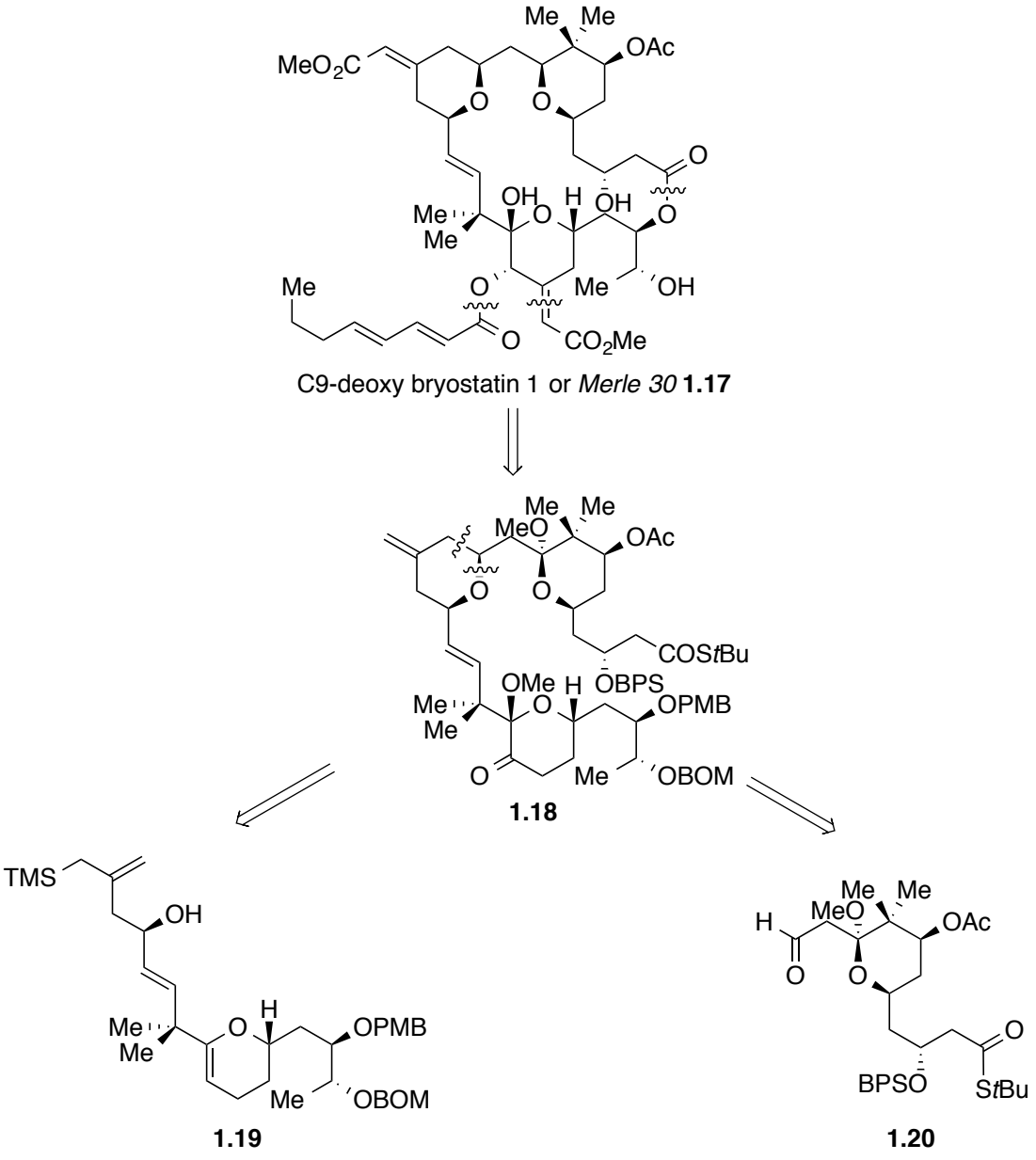


Figure 1.15. Retrosynthetic analysis of Merle 30

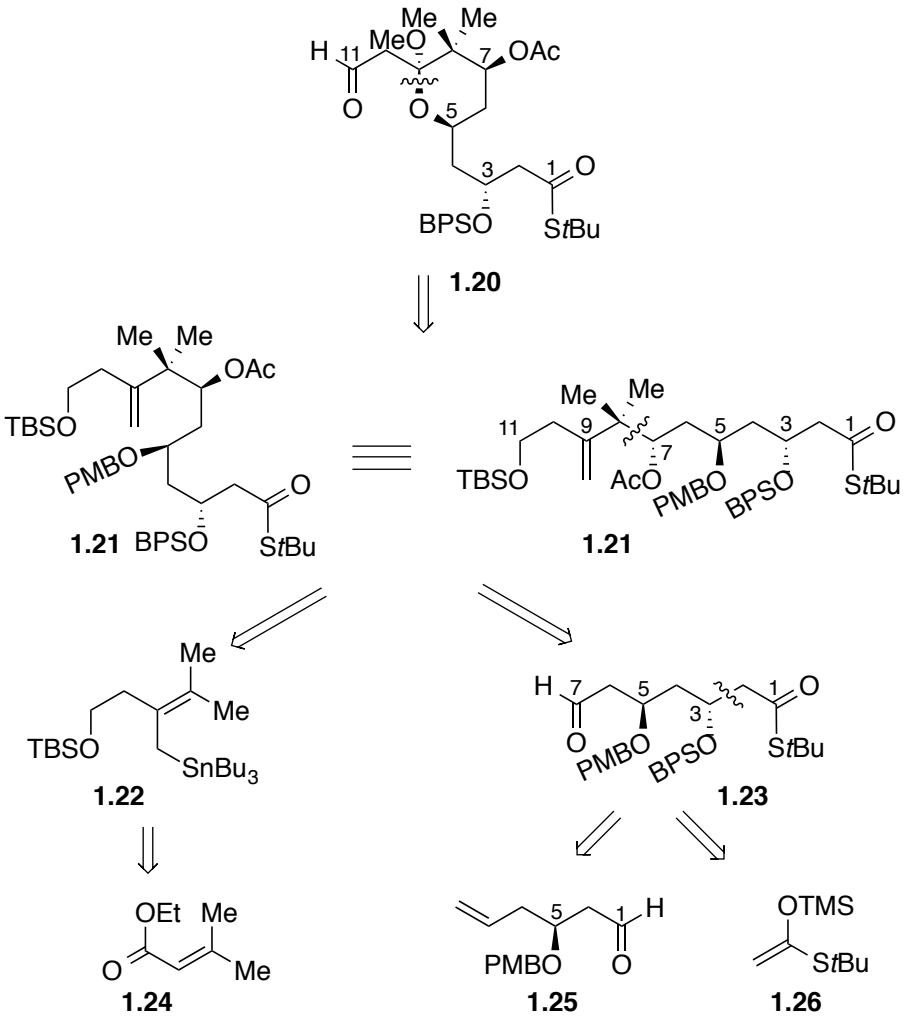


Figure 1.16. Retrosynthetic analysis for the A-ring

stannane **1.22** onto the aldehyde **1.23**. The stannane **1.22** could arise from 3,3-dimethyl acrylate. The aldehyde **1.23** could eventually be obtained from another chelation-controlled Mukaiyama aldol addition of the thioketene acetal **1.26** with the aldehyde **1.25**. Thus, we were left with one stereocenter at C5, which could be set using the Keck catalytic asymmetric allylation (CAA). This stereocenter at C5 could allow us to introduce the stereocenters at C3 and C7. The stereocenter at C7 would then eventually allow us to install the stereocenter at C9. This strategy could enable us to use our

synthetic methodology efficiently to introduce all the 4 stereocenters in the A-ring from a single chiral source of (*S*)-BINOL.

The C-ring β-hydroxyallyl silane was disconnected at C14-C15 to give the α,β-unsaturated aldehyde **1.27** and the trimethylsilylmethallyl tributylstannane **1.28** (Figure 1.17). We envisaged that a CAA reaction would install the desired stereocenter at C15. The aldehyde **1.27** could arise from a Horner-Wadsworth-Emmons olefination of an aldehyde derived from ozonolytic cleavage of the olefin **1.29**. The *gem*-dimethyl group at C18 was envisioned to arise from a prenylation of an aldehyde **1.30**. The aldehyde **1.30** could be installed using a hydroformylation reaction of the olefin **1.31**. The olefin **1.31** was disconnected at the C23-C24 bond, followed by another disconnection at the C24-C25 bond. Two consecutive chelation-controlled additions of allylstannane to the aldehyde derived from the commercially available lactate **1.32** should afford intermediate **1.31**.

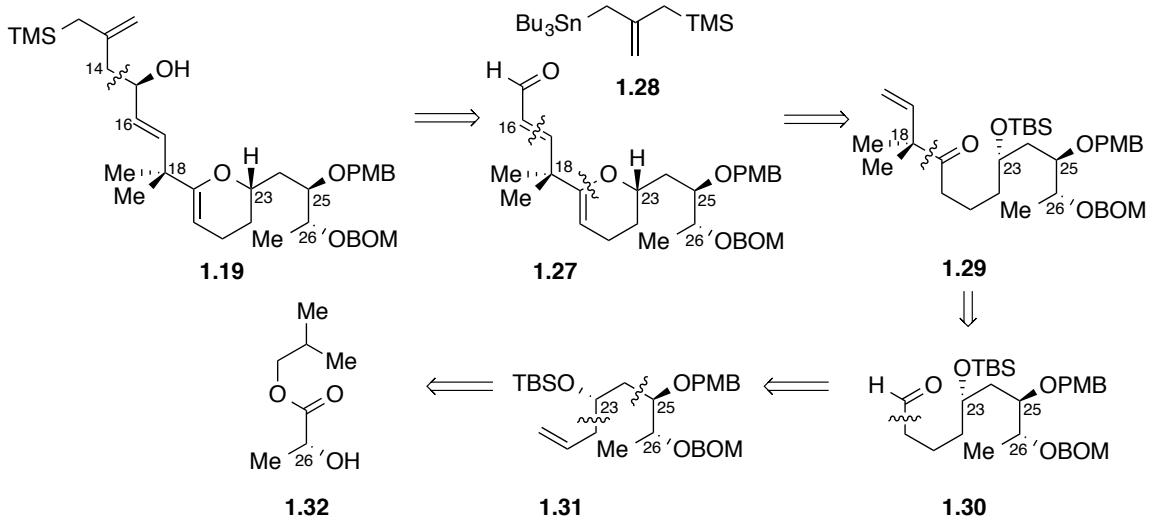


Figure 1.17. Retrosynthetic analysis of C-ring

Synthesis of A-ring aldehyde **1.20**

Dr. Dennie Welch, a former group member, originally developed the synthesis of the A-ring aldehyde **1.20**.[111] The synthesis began with the monoprotection of commercially available and cheap 1,3-propanediol **1.33** (Figure 1.18). Using 1 equivalent of *tert*-butyldimethylchlorosilane and an excess of the diol, the monoprotected alcohol **1.34** was obtained in high yield. Swern oxidation of the resulting alcohol gave the monoprotected aldehyde **1.35** in near quantitative yield.[112] The next step in the synthesis let us introduce the first stereocenter at C5 using CAA with allyltributyl stannane **1.36** and (*S*)-BITIP as the catalyst with high yield (99%) and excellent enantioselectivity (98% *ee*).[113] Scale up of this reaction to 65 grams proceeded well without any significant loss in yield and enantioselectivity. The next step was to protect the resulting homoallylic alcohol as a *p*-methoxybenzyl (PMB) ether **1.38**. The choice of PMB was deliberate, as

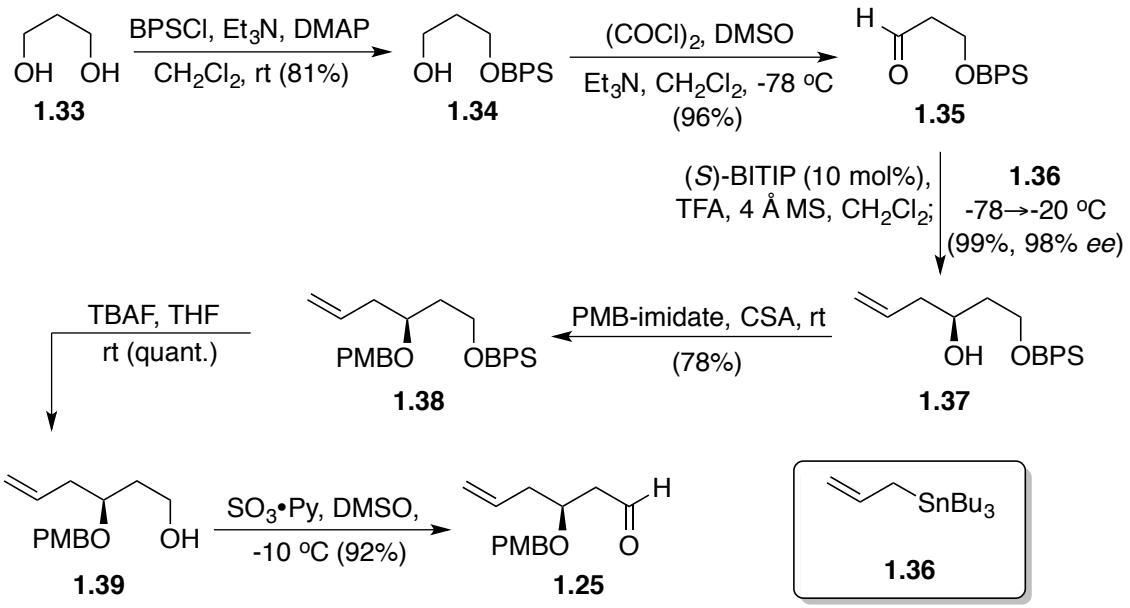


Figure 1.18. Synthesis of the aldehyde **1.25**

the chelating ability of PMB with common Lewis acids should aid in the formation of the C3 and C7 stereocenters in a substrate-controlled manner. Removal of BPS group with TBAF, followed by Parikh-Doering oxidation, gave the aldehyde **1.25** cleanly in very good yields.[114] Chelation-controlled Mukaiyama aldol addition with aldehyde **1.25**, using $Ti(OiPr)_2Cl_2$ as a Lewis acid, allowed us to introduce the C3 stereocenter with the desired 1,3-*anti* relationship as essentially a single diastereomer, yielding **1.41** (Figure 1.19). The *anti* relationship between C5 and C3 in **1.41** was established using Rychnovsky's acetonide method.[115] Compound **1.41** was subjected to TBS protection followed by reaction with DDQ to remove the PMB protecting group. Next the TBS protecting group was also removed to convert the diol into the acetonide **1.42** using 2,2-dimethoxypropane. During the removal of the TBS protecting group, the thioester was

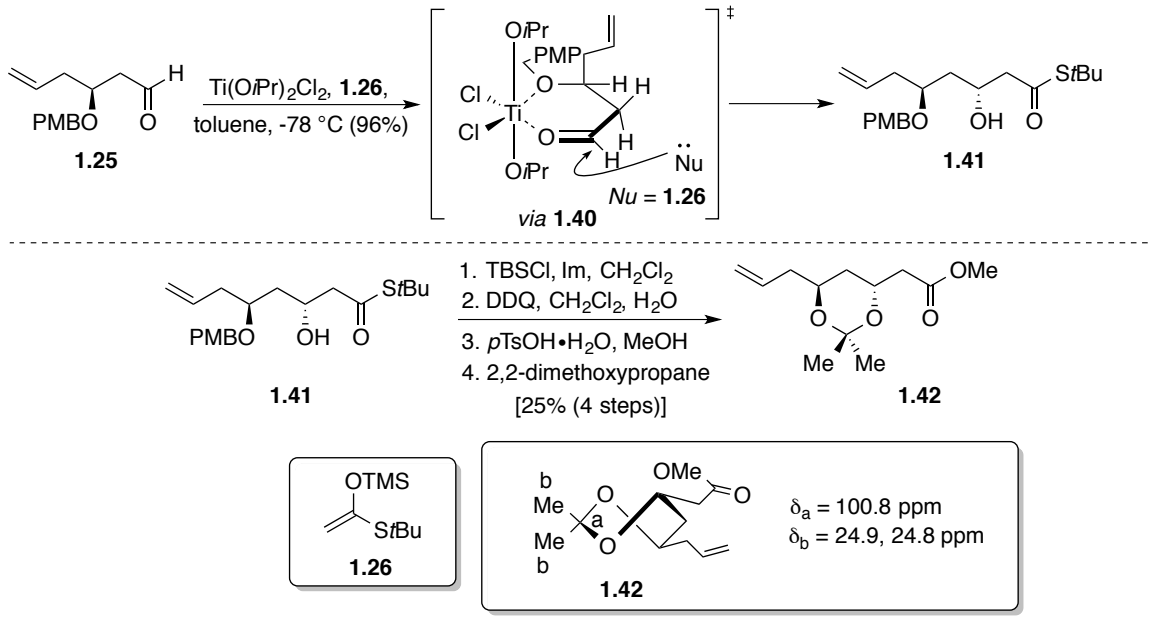


Figure 1.19. Stereoselective Mukaiyama aldol reaction and confirmation of stereochemistry

transesterified in the presence of methanol, which was not of any consequence for stereochemical studies. NMR analysis revealed that the ^{13}C chemical shifts of the geminal dimethyl groups and the carbon bearing the germinal dimethyls were indeed consistent with the expected chemical shifts reported by Rychnovsky assuming a twist boat type conformation **1.42**. Rationalization of the stereochemical outcome of this reaction came from an earlier work in our group, which proposed a transition state similar to **1.40**. In this transition state, steric hindrance prevents the nucleophile from attacking the aldehyde on the same face as that bearing the allyl group.[116] With the correct stereochemical relationship established, alcohol **1.41** was then protected with a BPS group, which is known to be nonchelating.[117] The selection of BPS was important at this point as we were about to take advantage of the C5 stereocenter again to introduce the next stereocenter at C7 and we were concerned that another chelating group at C3 would create some problems arising from competitive chelation. The required aldehyde **1.23** was obtained by the use of a catalytic amount of OsO_4 and NMO as co-oxidant and subsequent cleavage of the vicinal diol by $Pb(OAc)_4$ (Figure 1.20).[118] At this point we were ready to do another chelation-controlled addition on the aldehyde **1.23** to set the C7 stereocenter.

The synthesis of the stannane **1.22** for this addition began with the α-alkylation of

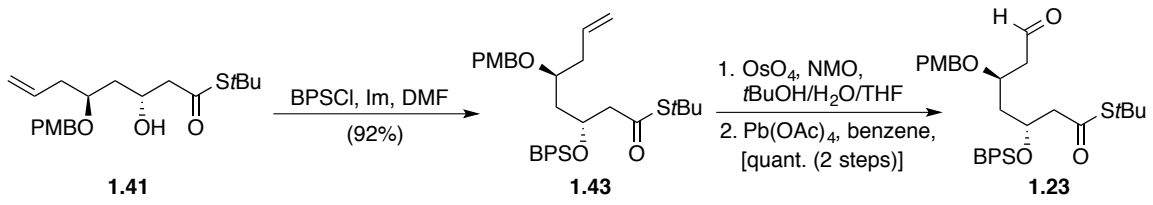


Figure 1.20. Synthesis of the aldehyde **1.23**

commercially available ethyl 3,3-dimethylacrylate **1.24** with 2-iodo-1-(*t*butyldimethylsilanoxy)ethane using LDA as a base (Figure 1.21). The next step was to isomerize the terminal olefin **32** to an internal olefin **1.26**. Interestingly, that olefin does not isomerize in the presence of the basic medium of the reaction mixture. Thus, the olefin was isomerized in a separate step using *t*BuOK as base to give α,β-unsaturated ester **1.27**. The solvent was degassed prior to use and utmost care was taken to prevent any introduction of oxygen into the reaction mixture, which can potentially oxidize the intermediate enolate.[119] The α,β-unsaturated ester **1.27** was reduced to the primary alcohol **1.46** using DIBAL-H. The hydroxyl group was then converted to a leaving group (mesylate), which was then displaced by tributyltin lithiate *in situ* to form the stannane **1.22**. The stannane was stable enough to be purified by column chromatography.

With stannane **1.22** and the aldehyde **1.23** in hand, we were successfully able to couple the two components in the presence of the chelating Lewis Acid Me_2AlCl to give the alcohol **1.48** as a single diastereomer in good yield (Figure 1.22). Not only did this

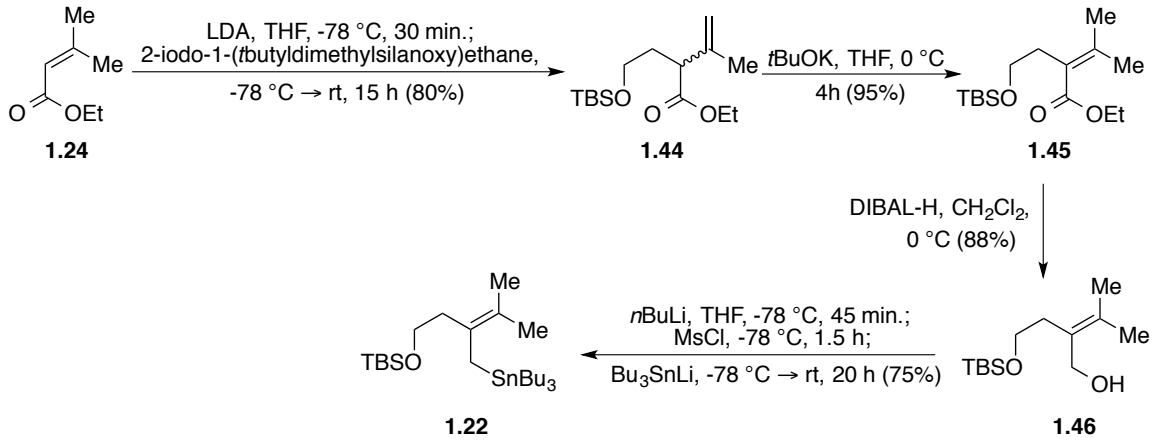


Figure 1.21. Synthesis of the stannane **1.22**

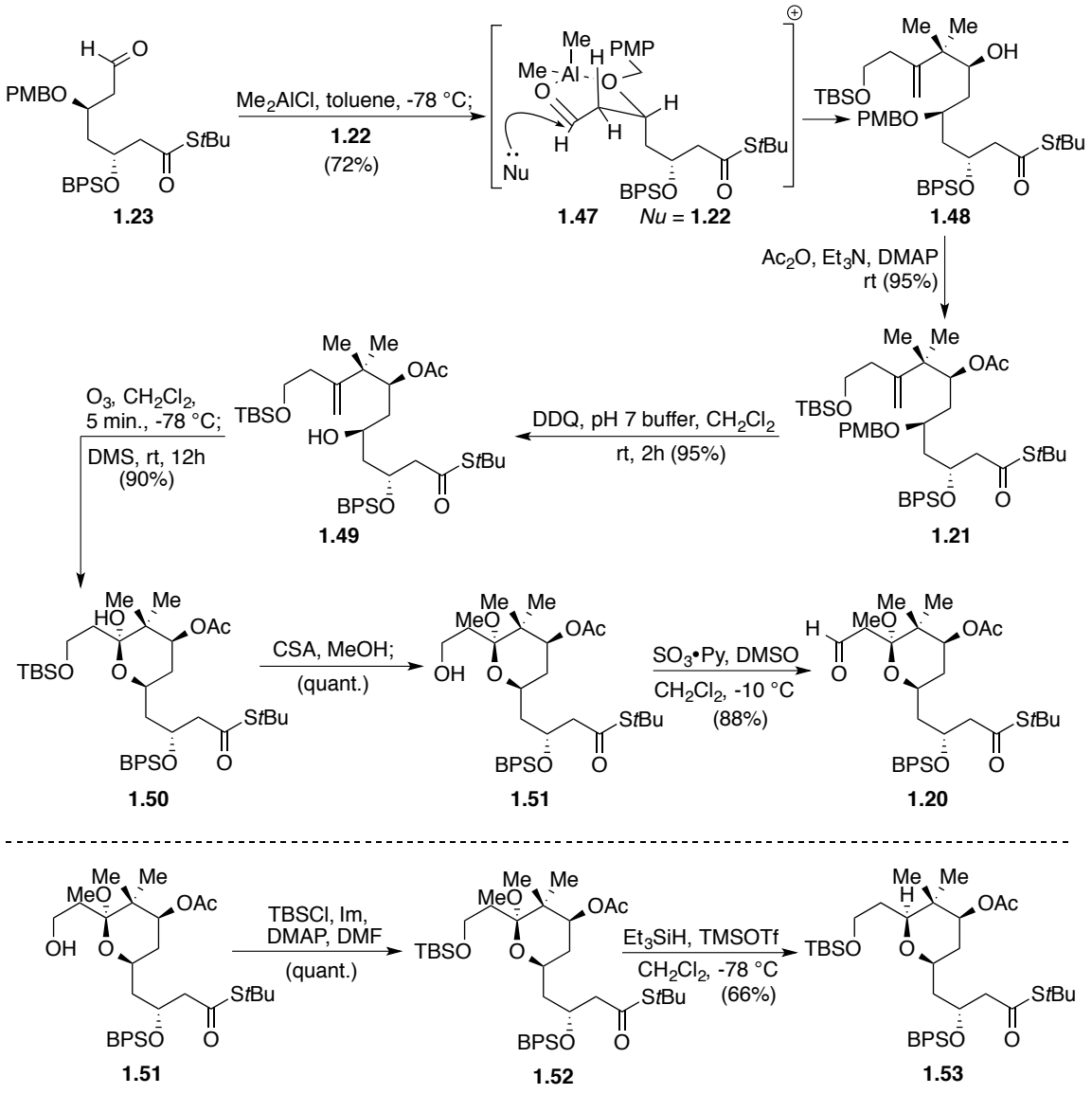


Figure 1.22. Synthesis of the aldehyde **1.20** and model studies for hydroetherification

step allow us to set the desired C7 stereocenter, but it proved to be an efficient way to install the C8 geminal dimethyl group as well. The Lewis acid solution was prepared freshly as it was found to be detrimental to use old stock solution. The stereochemical outcome of this reaction can be explained by a similar transition state like **1.40**. Additionally, Evans group had proposed a boat-like transition state **1.47** based on computational studies.[120] The PMB group was now deprotected using DDQ under pH 7 buffered reaction conditions in the presence of ample quantities of water.[121] Next, ozonolysis of the olefin resulted in exclusive formation of the cyclized hemiketal **1.50** without formation of any corresponding open chain isomer. Methanolysis of the hemiketal **1.50** in the presence of methanol and a catalytic amount of CSA gave the ketal **1.51** in quantitative yield. The acidic condition of the reaction medium also removed the terminal TBS group at C11.[122] Stereochemistry of the ketal was established by nOe studies by Dr. Dennie Welch in our group.[111a] The primary alcohol **1.51** was then oxidized under Parikh-Doering conditions to yield aldehyde **1.20** in good yield.

Model studies for deletion of the C9 hydroxy group

Before embarking on the journey towards the synthesis of Merle 30, we decided to use a model substrate to study the deletion of the C9 hydroxy group of bryostatin 1. The conditions developed in this transformation would then be used in a more advanced intermediate. The alcohol **1.51** was protected as TBS ether **1.52**. Acidic conditions with PPTS and Et_3SiH did not reduce the C9 ketal functionality. However, using TMSOTf as Lewis acid, we were able to perform the hydroetherification reaction smoothly to produce compound **1.53**.

Synthesis of the C-ring silane **1.19**

The route for the synthesis of the β-hydroxyallyl silane **1.19** was initially developed by Dr. Anh Truong and then scaled up by Mr. Jeffrey C. Stephens in our group.[123] Since this route was reported in reference no. 123, no detailed discussion will be given here until the synthesis of **1.29**. Please refer to the aforementioned reference, and Chapter 2 for a full discussion on a modified route to a fully functionalized C-ring intermediate. Present discussion will focus on synthesis of **1.19** starting from the olefin intermediate **1.29**. Ozonolysis of the olefin **1.29** followed by a reductive work-up produced the aldehyde **1.54**, which was immediately converted to the *trans*-α,β-unsaturated thioester **1.56** in very good yield using a Horner-Wadsworth-Emmons olefination with the phosphonate **1.55**. Removal of the TBS protecting group followed by a dehydrative cyclization gave the glycal **1.57** in very good yield. A selective half-reduction of the thioester **1.57** using DIBAL-H provided the aldehyde **1.27** (Figure 1.23). With the aldehyde **1.27** in hand, the next step (Figure 1.23) was to convert the aldehyde to the β-hydroxyallyl silane **1.19** using CAA. Dr. Yam Poudel from our group had previously attempted to reproduce the results of the CAA reaction performed by Dr. Anh Truong on the same substrate **1.27**. However, those results were not reproducible and he then attempted to synthesize the compound **1.19** in a step-wise fashion. The stannane **1.28** was added to the aldehyde in a nonstereoselective fashion by heating in toluene at reflux. Next, we oxidized the diastereomeric mixture **1.58** to form the ketone **1.59**. At this stage, we found a CBS reduction was successfully able to produce the desired silane **1.19** as a single diastereomer in very good yield. As the (*S*)-CBS reagent is not stable in THF solution beyond several days, we converted the catalyst to an adduct with borane to yield

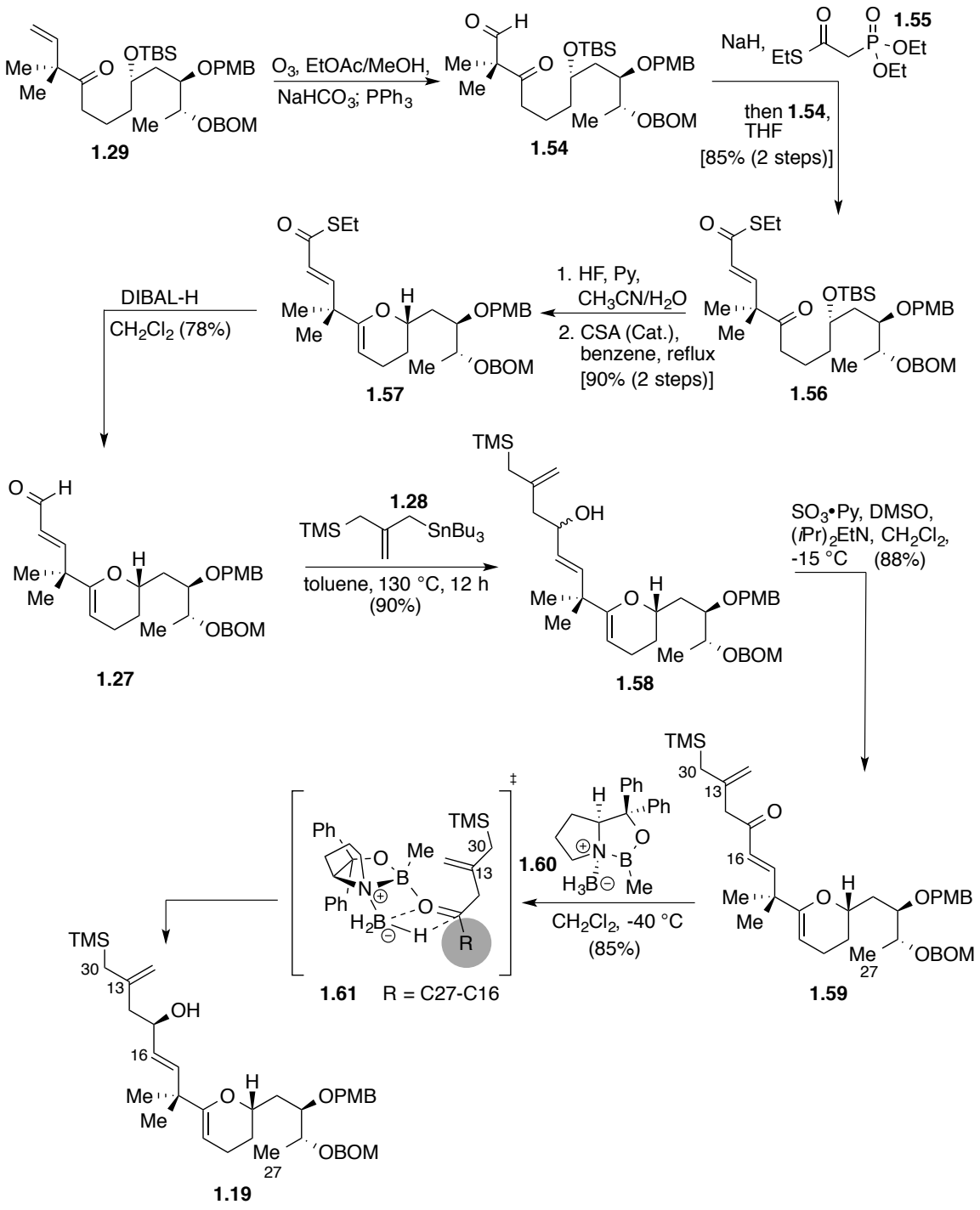


Figure 1.23. Synthesis of the C-ring silane **1.19**

a bench stable solid powder of (*S*)-CBS•BH_3 reagent **1.60**. The absolute stereochemistry of C15 was established by a Mosher ester analysis performed by Dr. Yam Poudel and can be explained by a transitions state like **1.61**.[124]

Completion of the synthesis of Merle 30

With the aldehyde **1.20** and the β-hydroxyallylsilane **1.19** in hand, our flagship reaction pyran annulation was applied to provide us with the tricyclic core of Merle 30 **1.62** in moderate yield (Figure 1.24). We were able to recover some TMS protected C-ring silane and unreacted A-ring aldehyde **1.20**. The TMS protected C-ring silane was converted back to C-ring silane **1.19** using mild acidic conditions with PPTS/MeOH. This intermediate **1.62** was prepared on about 300 mg scale and used for further functionalization towards Merle 30 and later Merle 32 (described later). Several attempts to improve the yield of this reaction such as varying temperatures, solvents, and order of addition did not result in significant progress. Use of various Lewis acids such as lanthanide metal triflates and silyl triflates (TBSOTf, TESOTf) either decomposed the substrates or protected the C-ring hydroxyallylsilane as silyl ethers. The steric environment around the bond-forming atoms in the pyran annulation reaction with compounds **1.19** and **1.20** seems to be the reason behind the moderate yield.

With the tricyclic core in hand, Dr. Yam Poudel focused on the functionalization of the C-ring glycal (Figure 1.24). A chemoselective epoxidation of the glycal using MMPP and subsequent epoxide opening with methanol *in situ* provided the ketal at C19 and a mixture of (1:1) diastereomers of alcohols at C20. This inconsequential mixture of diastereomers was converted to the ketone **1.18** using Ley oxidation conditions.[125] The

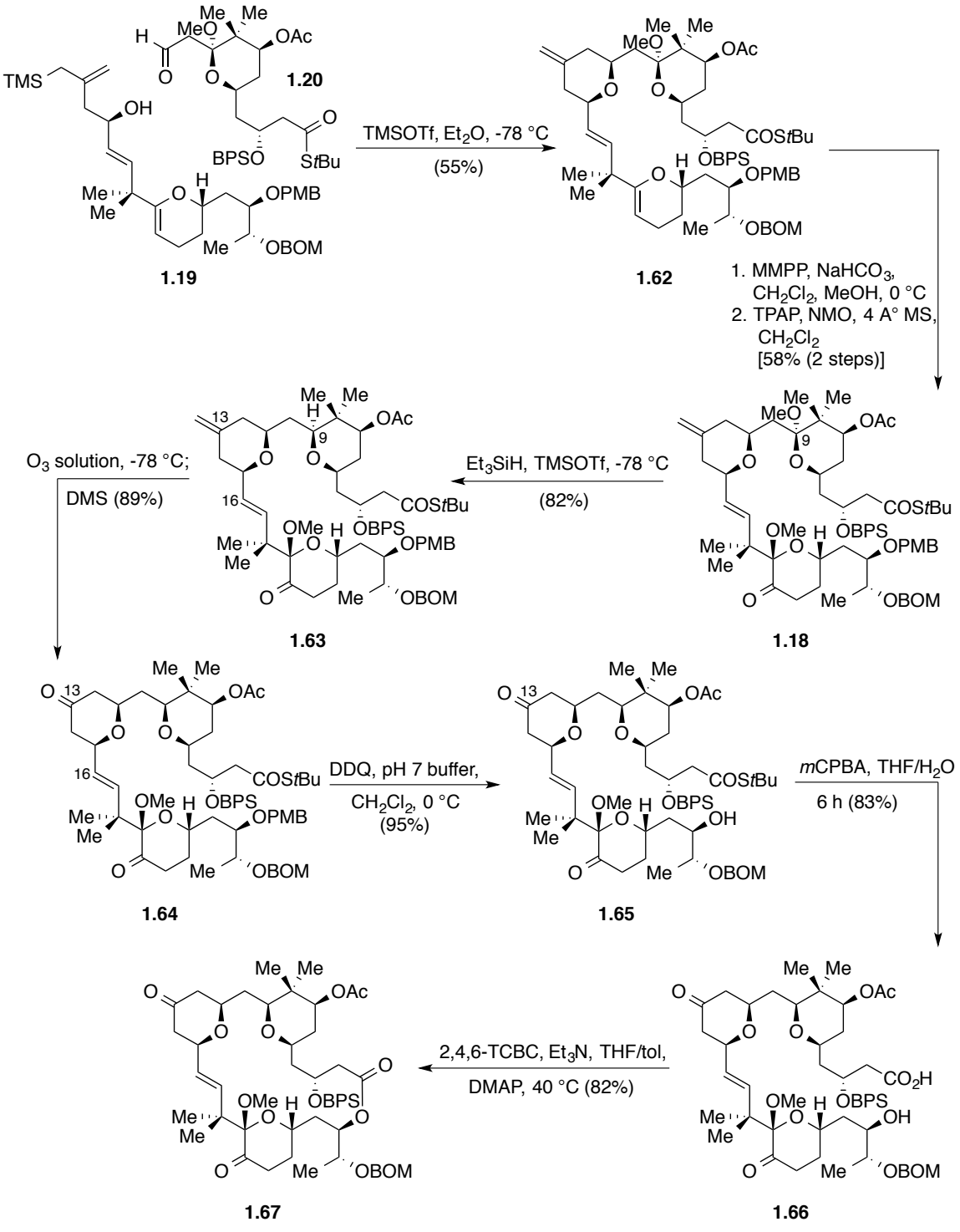


Figure 1.24. Synthesis of the macrolactone core for Merle 30

next step was to try the key reaction of deletion of C9 oxygenated functionality that we already successfully applied in a model substrate **1.52** (Figure 1.22). Although the ketone **1.18** contains two methyl ketals, the reactivity of the two functional groups is very different. The C-ring methyl ketal is located next to a ketone at C20 and the formation of an oxocarbenium intermediate using a Lewis acid would be energetically highly unfavorable. This unique situation provided an opportunity for a chemoselective reduction of C9 methyl ketal using triethylsilane and TMSOTf as the Lewis acid to provide **1.63**. The stereochemistry was proved using nOe correlations between C5, C7, and C9 hydrogen atoms. After exploring various choices of the sequences of steps and various optimizations, Dr. Yam Poudel found out that C13-C30 olefin on the B-ring needed to be oxidized to a ketone first in order to hydrolyze the thioester at C1. Additionally, the conversion of the ketone in the B-ring to the enoate functionality was not selective towards the desired *Z*-olefin with the open chain intermediates. A more conformationally constrained macrolactone would provide a better selectivity in the olefination using Fuji's BINOL. To achieve this, the olefin at C13 was regioselectively cleaved by drop-wise addition of saturated ozone solution to provide the diketone **1.64**. Next removal of the PMB group at C25, followed by hydrolysis of the thioester at C1, provided the seco-acid **1.66**. Yamaguchi macrolactonization on the seco-acid provided the macrolactone core **1.67**. The regioselective Horner-Wadsworth-Emmons olefination on the C13-C30 ketone using Fuji's chiral phosphonate **1.68** provided the B-ring enoate compound **1.69** in good yield and moderate diastereoselectivity (*Z*:*E* = 4:1) (Figure 1.25).[126] Stereochemical proof of the olefin geometry came from the nOe correlations between C30 proton and the equatorial proton on C12. The *Z*-isomer was isolated from

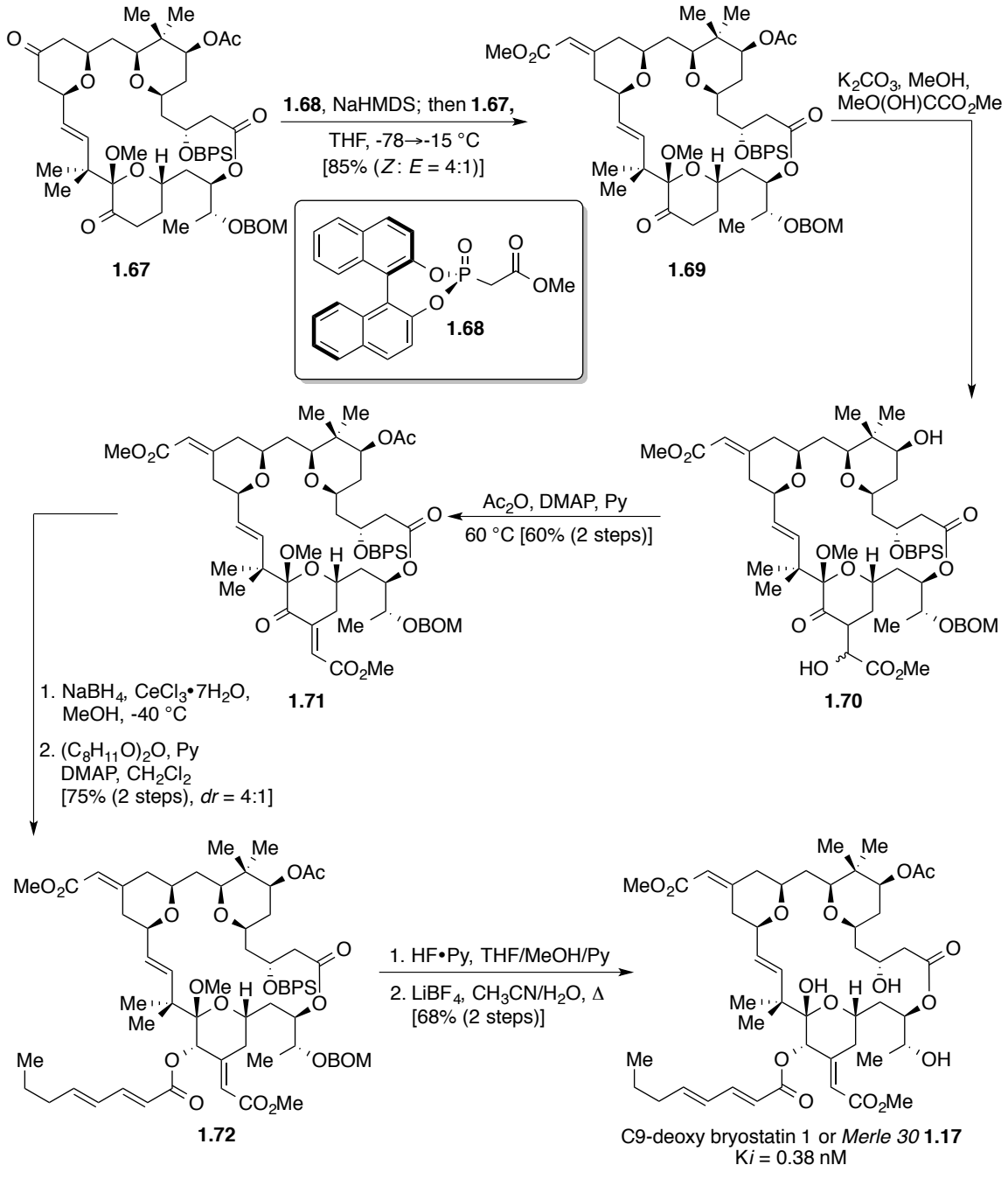


Figure 1.25. Completion of the synthesis of Merle 30

the *E*-isomer using preparative TLC.

The next step was to install the enoate on the C-ring (Figure 1.25). Dr. Yam Poudel after some optimizations was able to convert the ketone **1.69** to the alcohol **1.70** using K_2CO_3/MeOH and the methyl acetal of methyl glyoxylate. Heating the crude aldol product **1.70** with acetic anhydride and DMAP in pyridine provided the aldol condensation product **1.71**. A moderately selective Luche reduction followed by immediate esterification with octadieonic anhydride installed the bryostatin 1 side chain. The diastereomeric mixtures (*dr* = 4:1) were separated using another preparative TLC. Removal of BPS group followed by global deprotection using $LiBF_4$ afforded C9 deoxy bryostatin 1 or Merle 30.

Biological evaluation of C9 deoxy bryostatin 1 or Merle 30

Merle 30 was found to have an inhibitory dissociation constant (K*i* = 0.38 nM) comparable to that of bryostatin 1 (K*i* = 0.48 ± 0.03 nM) studied in mouse PKCα.[127] This suggested that the absence of H-bonding between the C9-OH and Met 239 of PKC backbone does not affect its binding affinity. This was not surprising given that other bryostatin analogues lacking the C9-OH have shown high affinity for PKCs (Figure 1.9). Functional activity of Merle 30 in living cells was assessed using the U937 cells and is shown in Figure 1.26 along with Merle 28. PMA showed strong antiproliferative responses, whereas bryostatin 1 caused only a minor, biphasic decrease in cell proliferation. When used in combination, bryostatin 1 was observed to block the anti-proliferative effect of PMA in a dose-dependent manner. Additionally, PMA induces attachment of the U937 cells whereas bryostatin 1 does not. Merle 30 exhibited largely

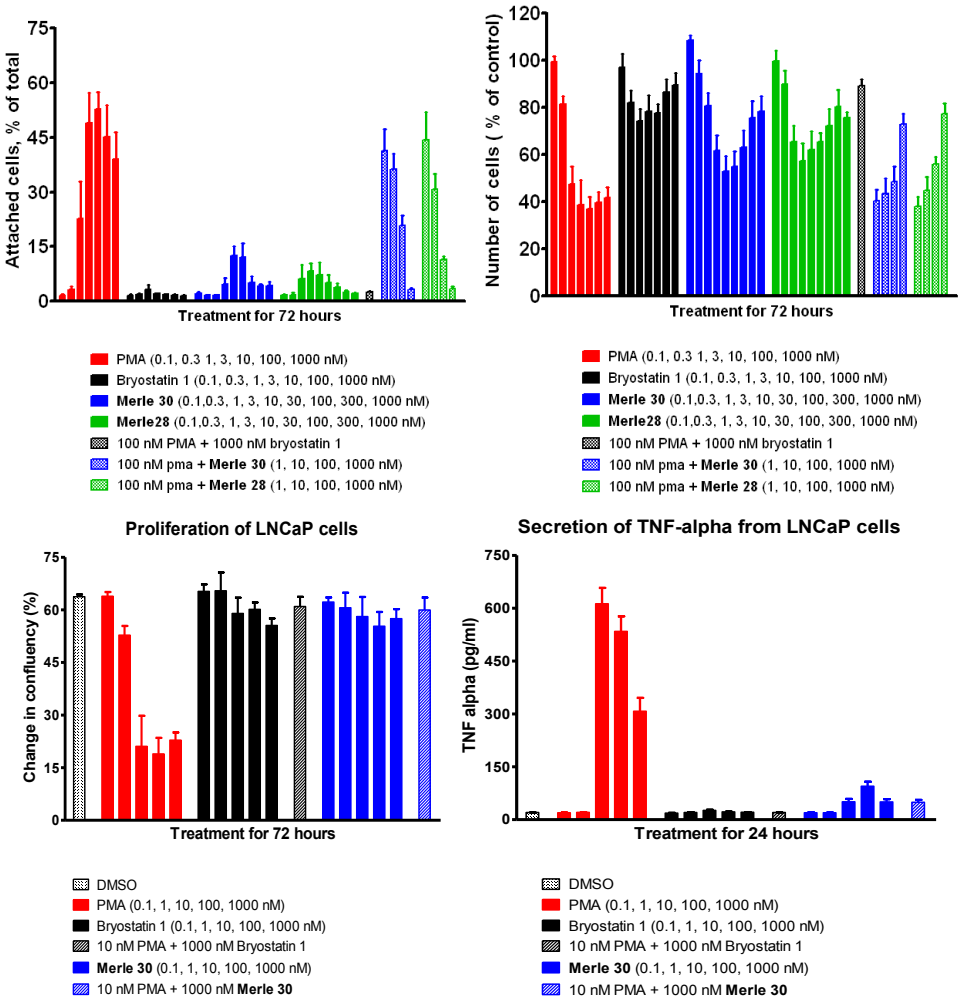


Figure 1.26. Biological profile of Merle 30

bryostatin–like biological responses. Interestingly, Merle 30 showed a little tilt towards PMA-like character with larger decrease in cell proliferation and greater attachment. Our studies with Merle 27, 28, and 30 revealed that deletion of C13 enoate, C9 hydroxy groups would provide more PMA-like character.

Merle 30 was also examined for proliferation and secretion of TNFα in LNCaP cells. PMA induces cell proliferation and induces apoptosis, whereas bryostatin 1 does not. Merle 30 did not inhibit proliferation but antagonized the inhibition by PMA. For

induction of TNFα secretion, the three agents behave differently. PMA induces a potent response while bryostatin has no response. Merle 30 induced a weak biphasic response. Additionally, those compounds which do not induce secretion themselves block the response to PMA. We also found a strong correlation between bryostatin-like patterns of gene expression and bryostatin-like patterns of biological response in U937 cells.[108] In conclusion, the C9 hydroxy group plays a minor role in both binding affinity and functional responses.

Synthesis of Merle 32

As mentioned earlier, the interactions between the lipid bilayer and the C1 domain bound to bryostatin are a key area to look into. The bryostatin northern hemisphere overlays the top face of the C1 domain, which interacts with the lipid bilayer. The combination of polar and nonpolar functionalities on the northern hemisphere plays a balanced role in the unique biological responses of bryostatin 1. So far with the syntheses of Merle 27, 28, and 30, we had individually scanned the roles of the C30 carbomethoxy group, the C9 hydroxy, and the C7 acetate group. Next, we focused on the role of the C8 geminal dimethyl group.

With the synthesis of Merle 30, we had already developed reactions to delete the C9 hydroxy group. We envisaged that the intermediate **1.63** could be utilized for further functionalization towards Merle 32 where all the polar functional groups would be deleted, leaving the C8 *gem*-dimethyl, and the two exocyclic olefins at C7 and C13. This would resemble Merle 23 except at the C8 position, which will also enable us to directly compare its biological properties with that of Merle 23.

The synthesis of Merle 32 (Figure 1.27) was developed by Dr. Yam Poudel and it commenced from the common intermediate **1.63**. The C25 PMB ether was deprotected using DDQ. Next, an oxidative hydrolysis using *m*CPBA enabled us to selectively hydrolyze C1 thioester in the presence of the C7 acetate. The reaction was stopped at 3.5 hours in order to prevent epoxidation of the C13-C30 olefin. Yamaguchi macrolactonization of the seco-acid **1.74** then furnished the macrolactone **1.75** in very good yield. To prevent competing enolization of the C7 acetate in the ensuing aldol reaction, the C7 acetate was removed using K_2CO_3/MeOH without any macrolactone opening at C1. The resulting free alcohol was then protected as TES ether **1.77**. The aldol reaction of the C-ring ketone **1.77** with freshly distilled methyl glyoxylate using LDA as base provided the aldol product, which was subjected to elimination using acetic anhydride to form α,β-unsaturated ester **1.78** as a single diastereomer. Luche reduction of the C20 ketone followed by an immediate esterification using 2,4-octadienoic anhydride provided the advanced intermediate **1.79** with a fully functionalized C-ring. Mild acidic conditions of PPTS/MeOH revealed the C7 alcohol by removing the TES group. Oxidation of the alcohol using Dess-Martin periodinane (DMP) provided the ketone **1.80** (Figure 1.28). Dr. Yam Poudel, after several attempts using various procedures, was finally able to convert the ketone **1.80** to the exocyclic olefin using Wittig conditions. Removal of the BPS group at the C3 position of **1.81** followed by global deprotection using $LiBF_4$ then completed the synthesis of Merle 32 **1.82**.

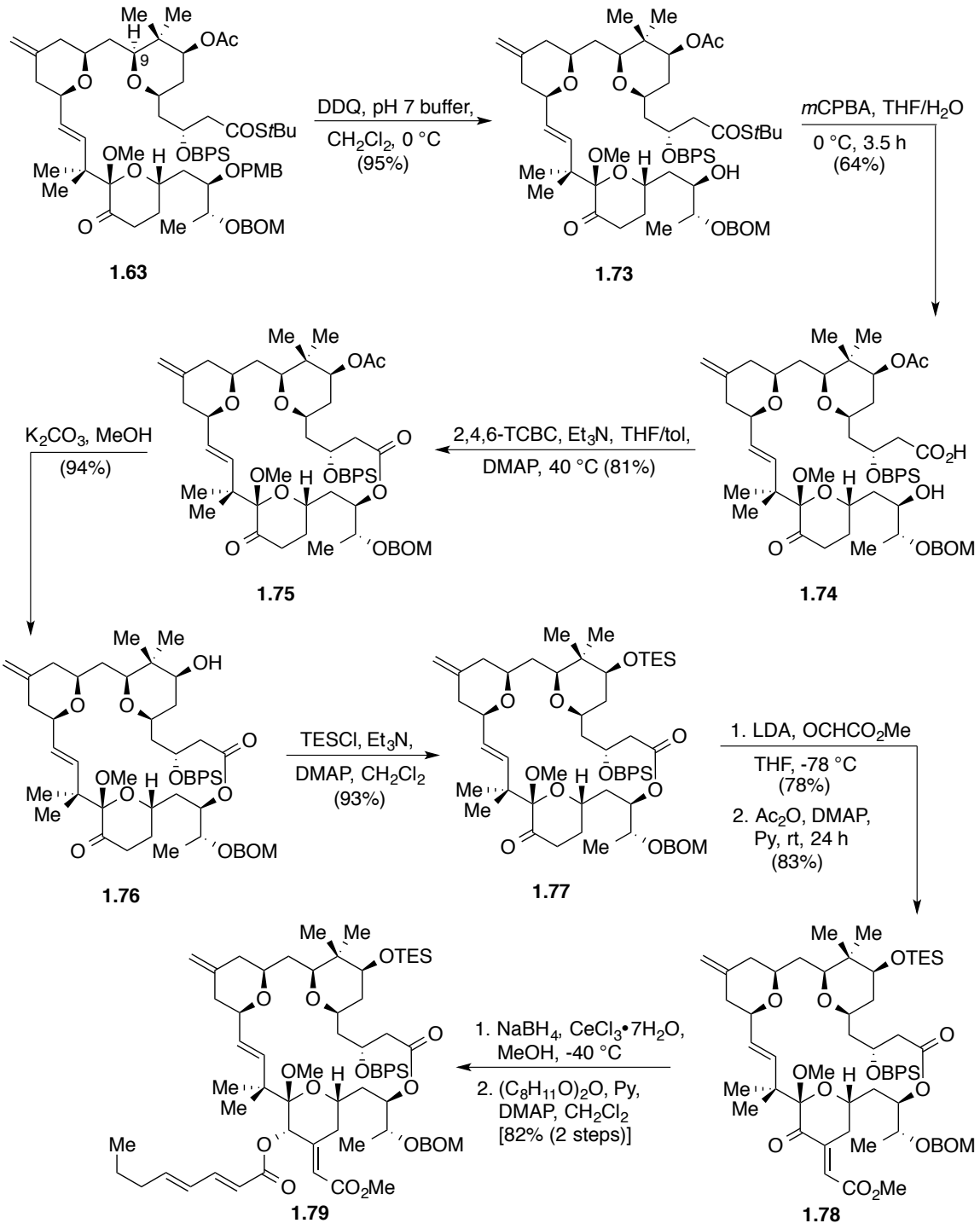


Figure 1.27. Synthesis of the advanced intermediate **1.79**

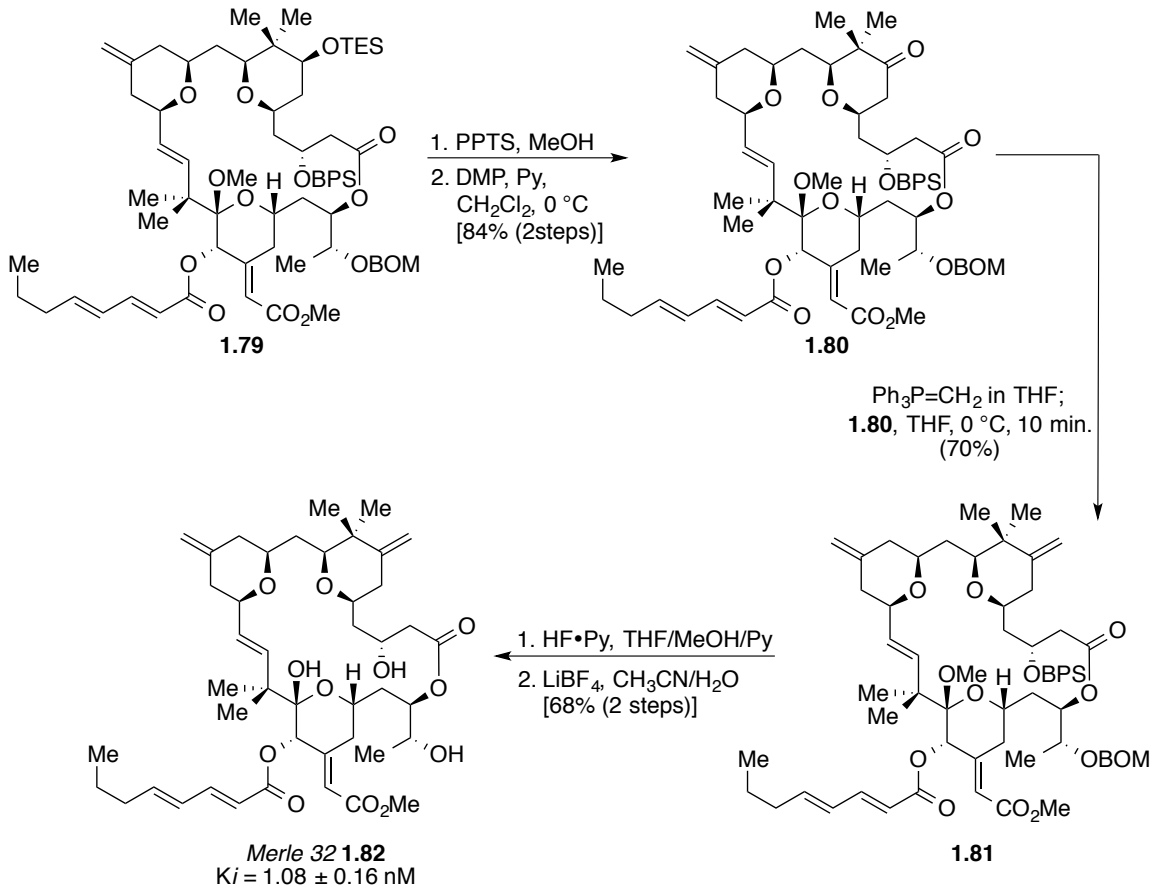


Figure 1.28. Completion of the synthesis of Merle 32

Biological evaluation of Merle 32

The biological evaluation of Merle 32 began with the determination of its binding affinity (K*i*) towards PKC *in vitro*. Its K*i* (1.08 ± 0.16 nM) proved comparable to bryostatin 1 (K*i* = 0.48 ± 0.03 nM) and to other bryopyran analogues in the Merle series. In the proliferation and attachment assays in U937 cells, Merle 32 resembled PMA and not bryostatin 1 (Figure 1.29 and 1.30). PMA induces attachment and inhibits proliferation, whereas bryostatin 1 has little effect. Bryostatin 1 blocks both responses to PMA in a dose-dependent manner when both the agents are applied together. Merle 32 retained PMA-like responses in different cell lines such as K562 and MV4-11 human

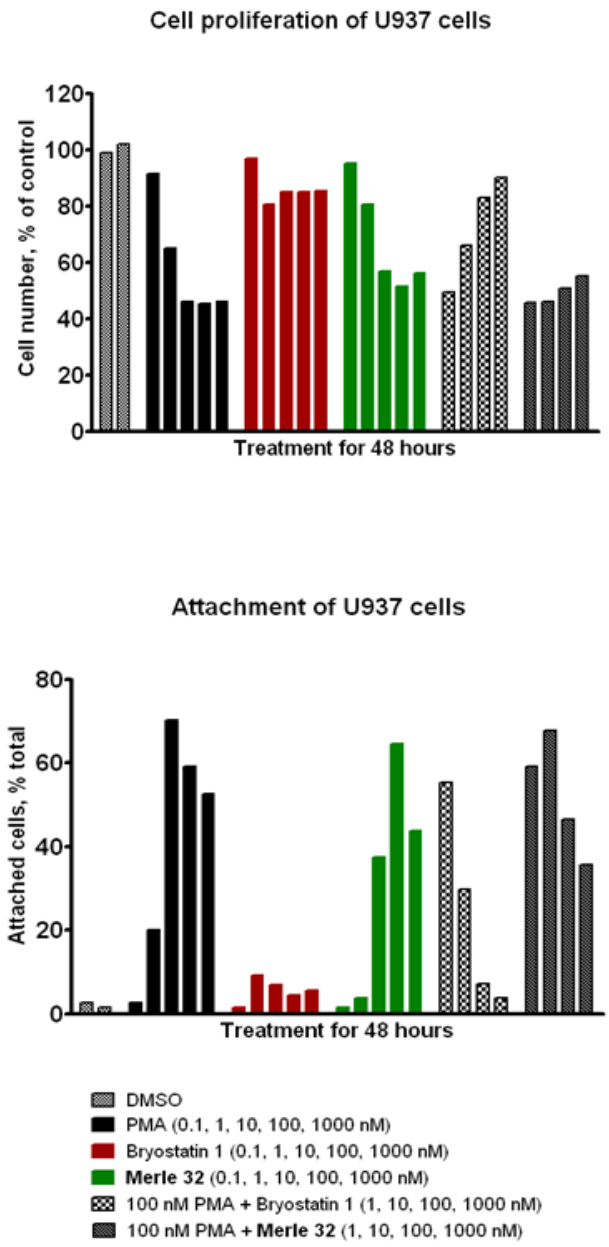


Figure 1.29. Attachment and proliferation assays with U937 cells and Merle 32 responses

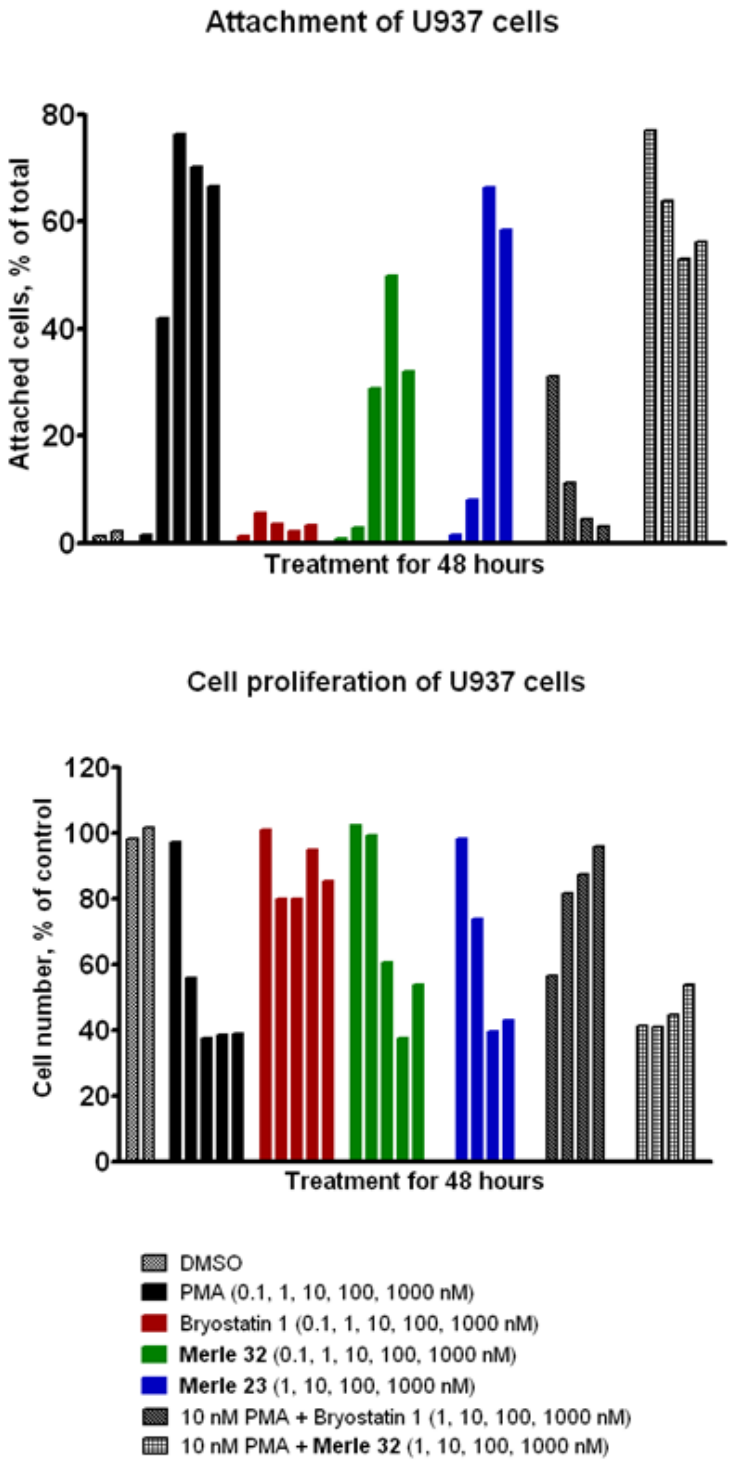


Figure 1.30. Comparison between Merle 23 and 32

leukemia cell lines.[128] Even with the human prostate cancer cell line (LNCaP), Merle 32 induced attachment and inhibited proliferation (Figure 1.31). From all these assays, it was clear that the C8 gem-dimethyl group in Merle 32 was not solely responsible for the unique biology of bryostatin 1. Comparison with Merle 23 corroborated this observation clearly (Figure 1.30).

Downregulation of PKC isoforms and other C1 domain-containing proteins after ligand binding was also studied with Merle 32.[128] Dose-dependent patterns of downregulation were determined for PMA, bryostatin 1, and Merle 32 in K562 cells. Bryostatin 1 was more effective in downregulating PKCα and β and showed a biphasic downregulation with PKCδ. Additionally, it did not cause the prominent induction of PKCε and RasGRP3 observed in these cells with PMA. Merle 32 showed a pattern very similar to that of PMA but with less potency. Another assay monitored the response of LNCaP cells towards TNFα secretion on exposing the cells to the Merle compounds in the presence of Lactacystin. Lactacystin is a proteasome inhibitor, which prevents the downregulation of PKC. Merle 28, 30, and 32 switched to display PMA-like properties in the presence of Lactacystin. These results also suggested that the properties of the Merle compounds could be reverted to more PMA-like (Figure 1.32).

Comparison of Merle 30 and 32 showed that they behaved differentially in U937 cells. Additionally, in MV-411 cells and K562 cells, Merle 30 and 32 showed similar contrasting responses. Merle 32 inhibited proliferation and induced attachment in MV-411 cells, similar to PMA (Figure 1.33). Merle 30 seems to have an effect intermediate between bryostatin 1 and PMA in the proliferation assay in K562 cells (Figure 1.34). This showed that in certain cells, Merle 30 could behave somewhat differently than bryostatin

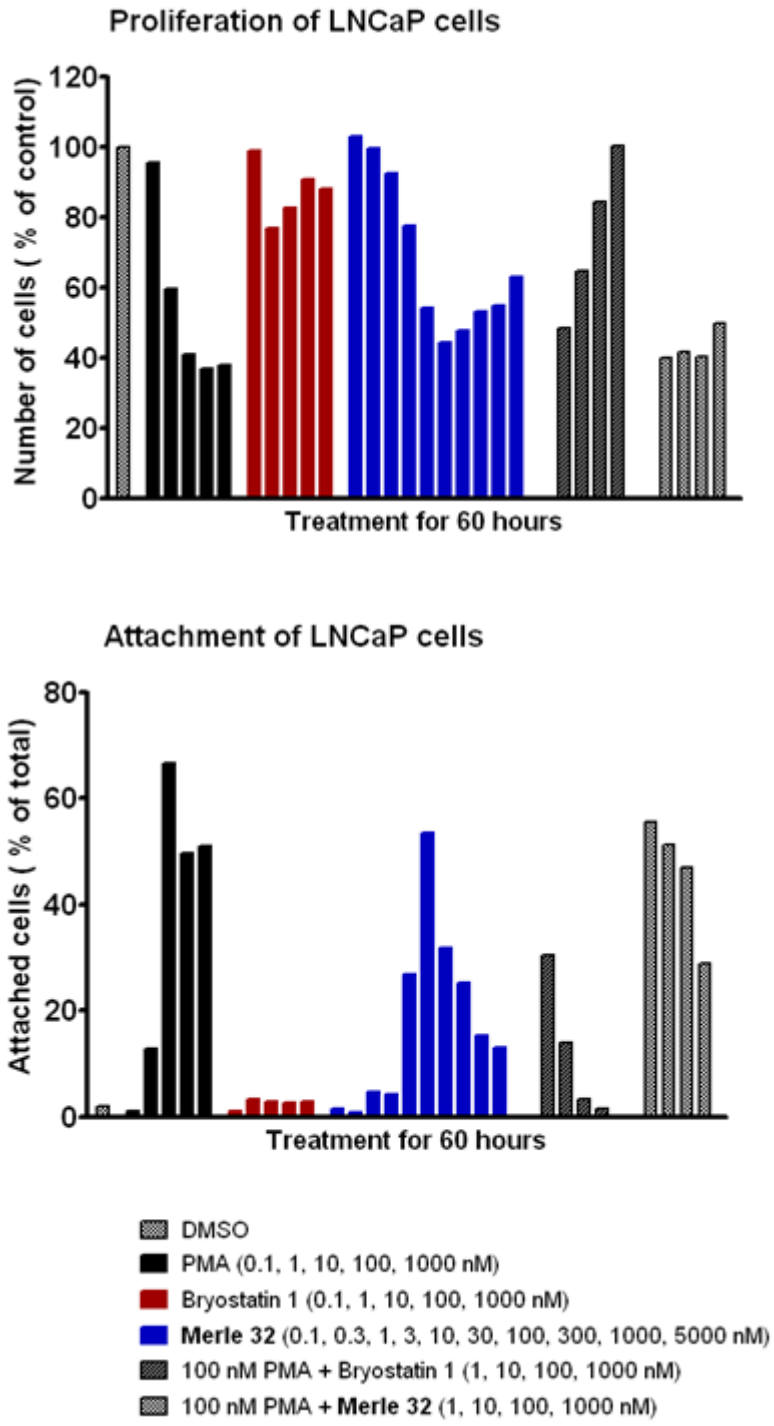


Figure 1.31. Responses of Merle 32 in LNCap cells

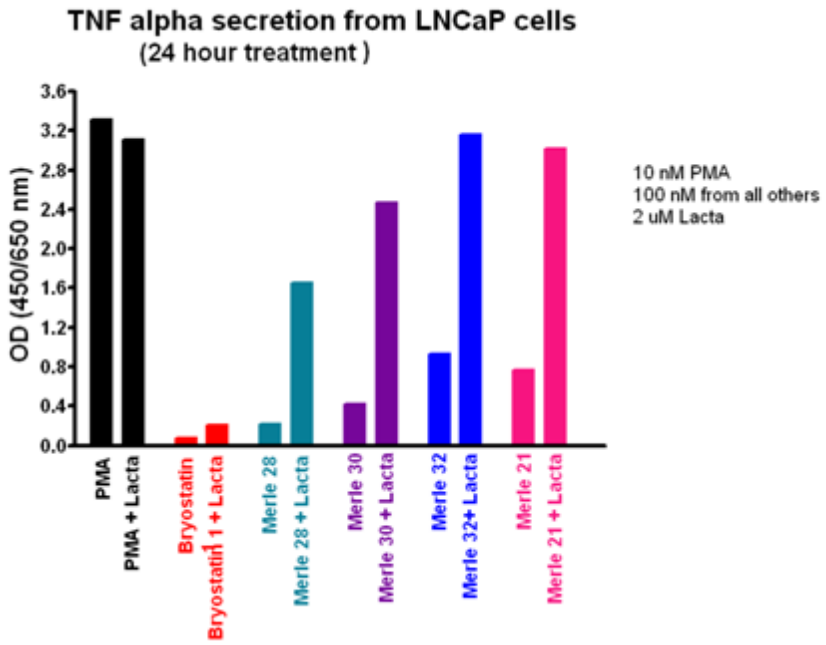


Figure 1.32. Reversal of the biological responses of various analogues

1 and could start resembling PMA.

In addition to the cell attachment and proliferation assays, the analogues were also tested by examining the translocation of PKCδ. LNCaP cells were transfected with GFP-PKCδ and then treated with the indicated molecules. The translocation of GFP-PKCδ was detected by confocal microscopy in real time with images taken every 30 s. The images shown (Figure 1.35) are representative of those from three independently performed experiments. The most lipophilic PKC ligands induce translocation of GFP-PKCδ mostly to the plasma membrane, whereas less-lipophilic PKC ligands, including bryostatin 1 and Merle 28, induce translocation to internal membranes. The more lipophilic compounds Merle 23, 32, and PMA showed clear similarity in translocating PKCδ to the plasma membrane. Merle 30, interestingly, was in between PMA and bryostatin 1 in translocating PKCδ.

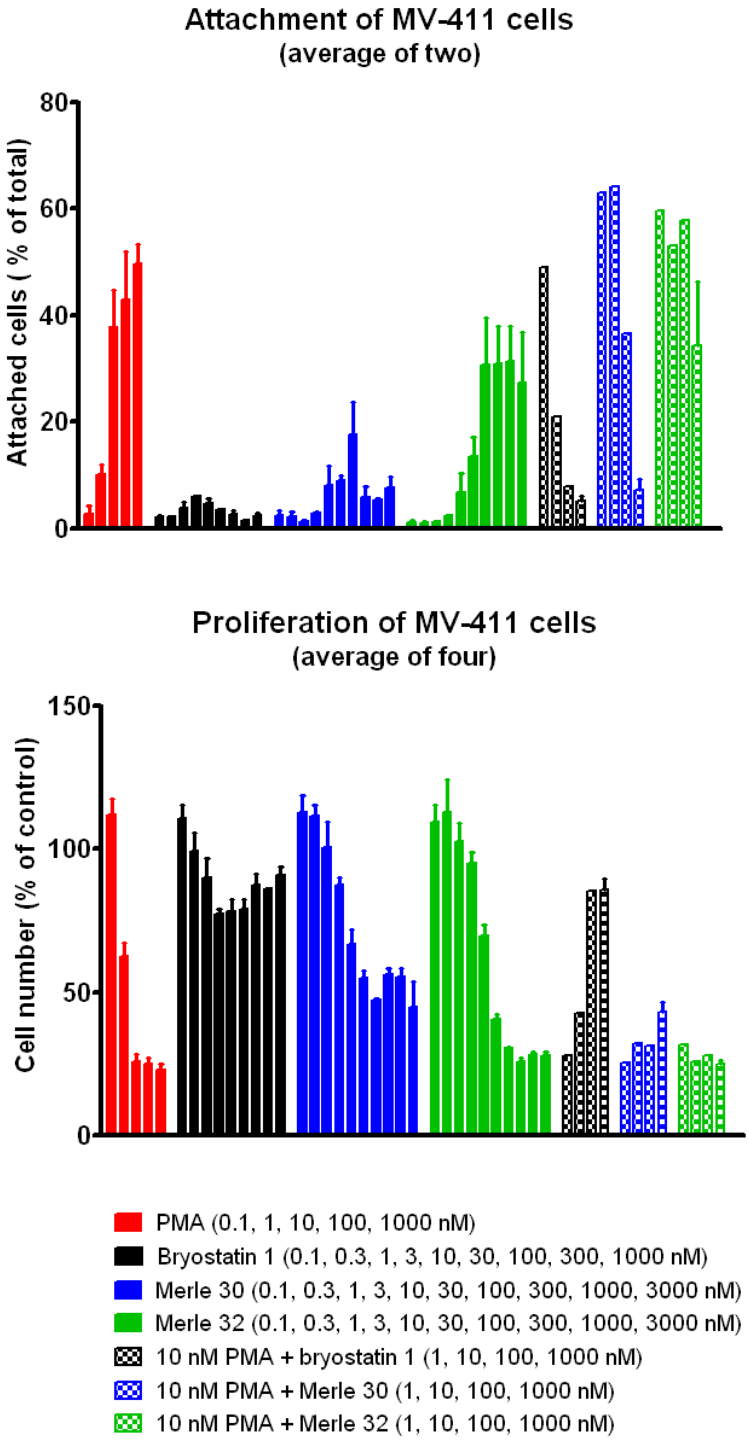


Figure 1.33. Comparison between Merle 30 and Merle 32

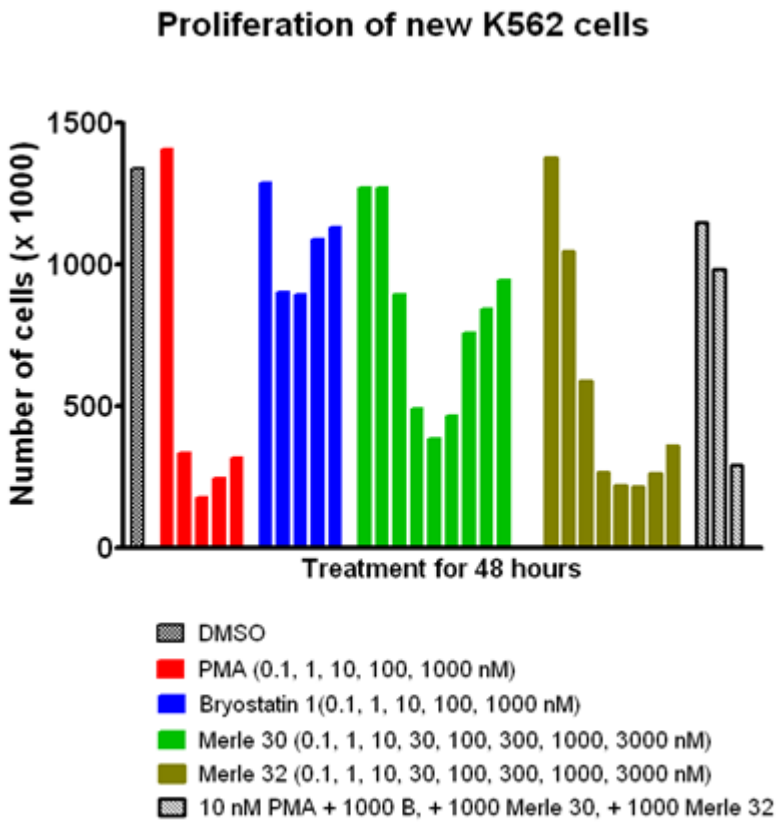


Figure 1.34. Proliferation responses of Merle 30 and 32 in K562 cells

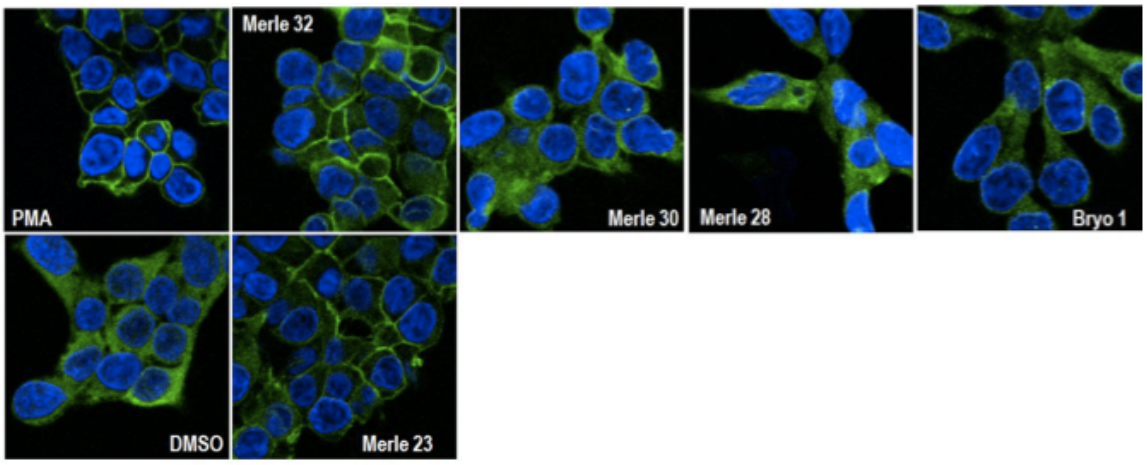


Figure 1.35. Translocation of PKCδ after 2 h treatment of LNCaP cells

Total synthesis of bryostatin 1 and 7

The retrosynthetic plan for the syntheses of Merle 30 and 32 explored the possibility of a convergent route via the intermediates **1.19** and **1.20** (Figure 1.15). Although that route was used successfully in the syntheses of Merle 28, 30, and 32, several problems in the route posed serious challenges while scaling up. Some of the problems included simultaneous aldol reaction on the C7 acetate during the late stage functionalization of C-ring and low selectivity during Luche reduction of the C20 ketone in advanced intermediates. Additionally, the retrosynthetic analysis of the C-ring silane **1.19** was essentially linear in nature and hence, the synthesis was not very efficient (Figure 1.17). At this stage Thomas Cummins in our group explored a more convergent retrosynthetic analysis of C-ring. In this retrosynthesis, we also explored the idea of a disconnection that would produce synthetic equivalents of A-ring β-hydroxyallyl silane **1.84** and C-ring aldehyde **1.83** (Figure 1.36). These two moieties are equally complex and we envisioned that they could be coupled using pyran annulation. The other disconnection was at the macrolactone ester linkage, which could arise from Yamaguchi macrolactonization. The A-ring β-hydroxyallyl silane **1.84** could arise from the same aldehyde **1.20** prepared earlier for the syntheses of Merle 30 and 32.

The C-ring enoate was thought to arise from the ketone **1.85** through an aldol condensation with methyl glyoxylate. The installation of the natural side chain of bryostatin 1 would happen after the selective reduction of the C20 ketone of aldol condensate. We imagined that the ketone **1.85** would arise from sequential oxidation reactions on the glycal **1.86**. The dihydropyran ring of **1.86** was envisioned to arise from Rainier modified Takai-Utimoto olefinic ester cyclization of **1.87**. The obvious

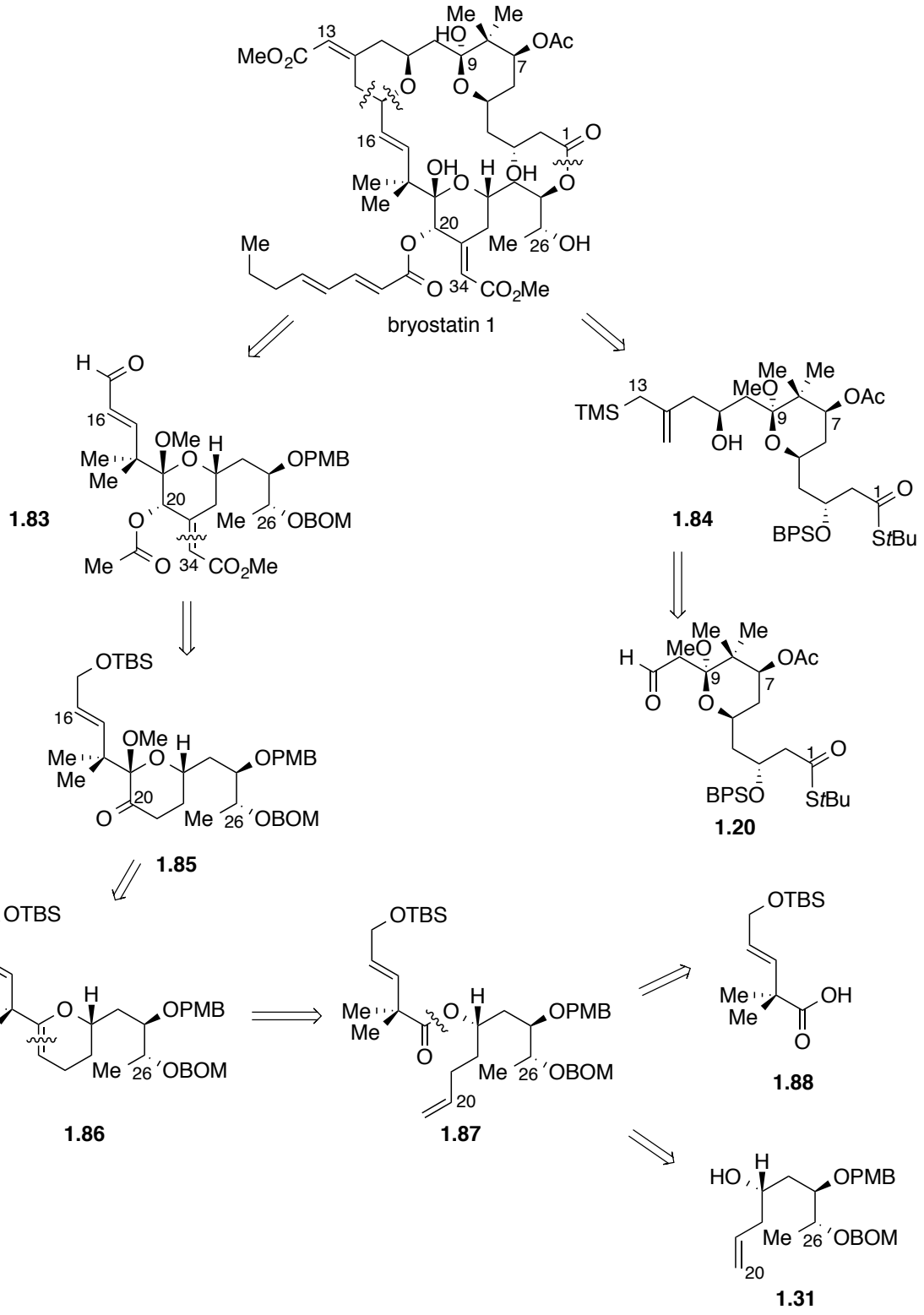


Figure 1.36. Revised retrosynthesis of bryostatin 1

disconnection at the ester linkage of **1.87** would then give the synthetic equivalents of the carboxylic acid **1.88** and the known secondary alcohol **1.31**.[123a] The carboxylic acid **1.88** could be prepared from commercially available methyl isobutyrate in four steps and the alcohol **1.31** could be obtained from commercially available (*R*)-isobutyl lactate.

Synthesis of the A-ring β-hydroxyallyl silane **1.84**

Synthesis of the A-ring silane **1.84** began from the previously prepared aldehyde **1.20**. Previous efforts to prepare a similar silane were performed by Dr. Dennie Welch, but the synthetic route failed to provide the desired silane.[111b] Dr. Yam Poudel attempted to perform the CAA reaction with the silane **1.28**, but no product was observed in this reaction, possibly due to the sterically hindered nature of the aldehyde **1.20**. Another alternative approach was to add the stannane **1.28** to the aldehyde **1.20** with no diastereoselectivity. This inconsequential mixture of diastereomers was then oxidized to the ketone **1.90**. After screening several reducing agents in presence of Lewis acids, Yam was able to prepare the β-hydroxyallyl silane **1.84** in moderate selectivity. The stereochemistry of the major diastereomer was proved via chemical transformations and comparing NMR spectra.[124] The most likely explanation for the diastereoselectivity comes from a half chair chelate structure **1.91** where the hydride nucleophile attacks from the bottom face, which leads to a chair-like transition state rather than a high-energy twist boat type transition state in case of attack from the top face (Figure 1.38).

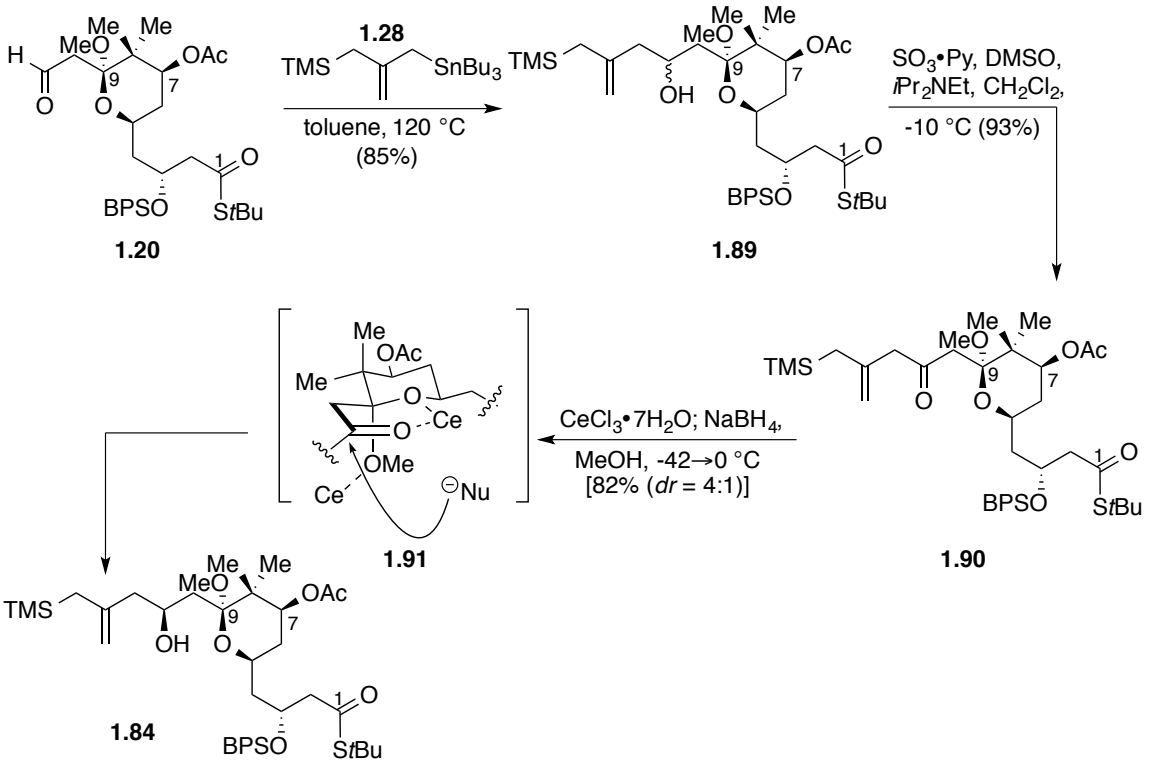


Figure 1.38. Synthesis of the β-hydroxyallyl silane **1.84**

A convergent synthesis of the C-ring aldehyde **1.83**

Synthesis of the carboxylic acid **1.88** began with the alkylation of commercially available methyl isobutyrate **1.92** followed by a free radical bromination (Figure 1.39). The resulting 4-bromoester **1.93** was then treated with *t*butyldimethylsilanol in the presence of silver triflate and a base to displace the bromide. Basic hydrolysis of the methyl ester furnished the carboxylic acid **1.88**.

Synthesis of the secondary alcohol **1.31** using a series of 1,2 and 1,3 chelation-controlled allylations has been reported earlier from our group and will not be discussed here.[123a] The carboxylic acid **1.88** was coupled with the alcohol **1.31** to provide the ester **1.94** (Figure 1.40). The ester **1.94** was then subjected to hydroboration and oxidation

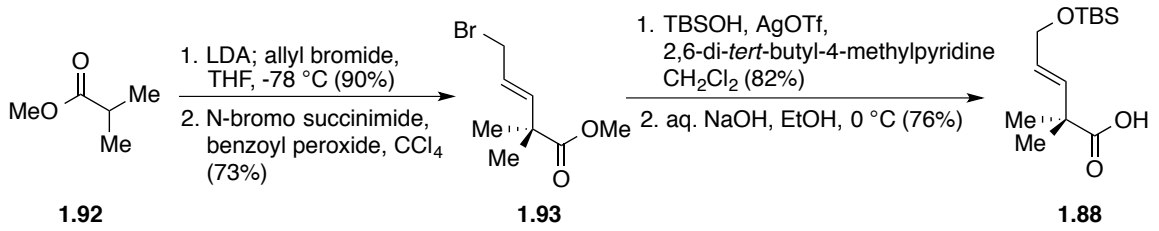


Figure 1.39. Synthesis of the carboxylic acid **1.88**

to provide the terminal alcohol **1.95**, which was then converted to an aldehyde using Parikh-Doering oxidation. A one-carbon homologation using a Wittig reaction produced the terminal olefin **1.87**. With the olefinic ester **1.87** in hand, we then subjected it to the Rainier-modified Takai-Utimoto reaction also known as Rainier metathesis.[129] To our delight, Thomas was able to convert **1.87** to the glycal **1.86** in a very good yield. $PbCl_2$ is a necessary additive to facilitate this reaction, as reported earlier by Takai and coworkers.[130] Use of ethyl bromide replacing methyl bromide was observed to produce exclusively the cyclic enol ether following Rainier's procedure. Epoxidation of the glycal using MMPP followed by *in situ* opening with methanol furnished a ketal at C19 and an inconsequential mixture of diastereomers at C20, which was immediately oxidized to the ketone **1.85** using Ley oxidation. An aldol condensation between the ketone and freshly distilled methyl glyoxylate provided the α,β-unsaturated ester **1.96** as a single geometrical isomer in excellent yield. The ketoester was then reduced to the alcohol using Luche conditions and then immediately esterified using acetic anhydride. The TBS group was removed to form the primary allylic alcohol, which was then oxidized to the aldehyde **1.83**. The choice of acetate as the protecting group in **1.97** was preplanned. Yam Poudel had previously found that the hydrolysis of the C7 acetate using K_2CO_3/MeOH conditions was at least 5 times faster for the C20 acetate in an advanced intermediate **1.75**

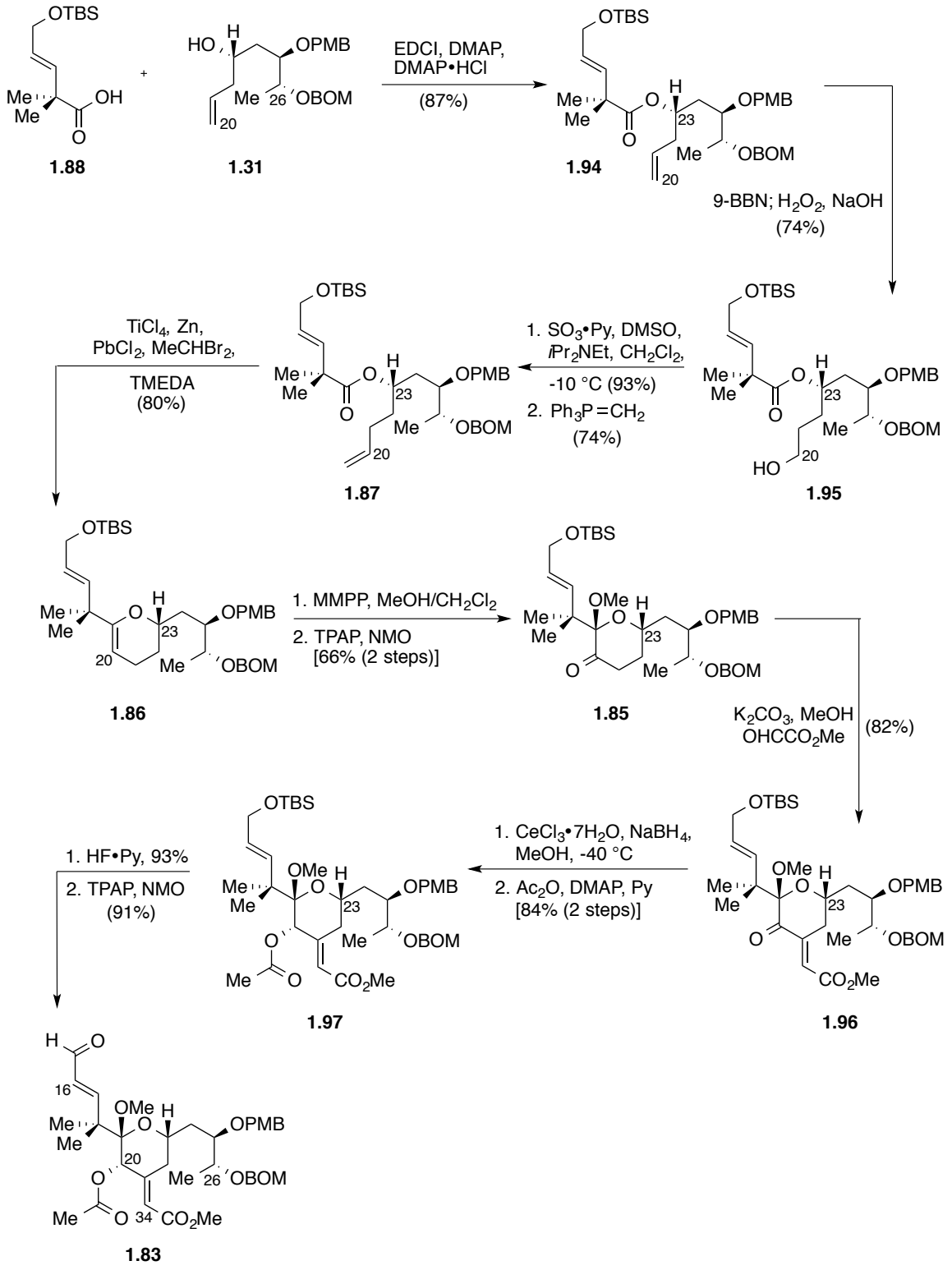


Figure 1.40. Completion of the synthesis of the C-ring aldehyde **1.83**

en route to Merle 32. This observation encouraged us to pursue bryostatin 7 using the same synthetic route. Additionally, this would also allow us to install various ester functionalities at a late stage during the synthesis of bryostatin analogues.

Completion of the synthesis of bryostatin 1

With both fragments in hand, A-ring hydroxyallylsilane **1.84** and C-ring aldehyde **1.83** were subjected to the crucial pyran annulation reaction (Figure 1.41). The reaction provided the tricyclic core **1.98** in moderately good yield. The major by-product observed by Dr. Yam Poudel was a spirocyclization, which resulted from an intramolecular cyclization of the hydroxyallylsilane to the C9 position. Hydrolysis of the thioester at C1 was not successful with the BPS group on C3, possibly due to steric hindrance. This prompted us to remove the C3 BPS group, which allowed us to hydrolyze the thioester followed by the protection of the C3 hydroxy group with TES. The presence of the free hydroxy group at C3 can form a hydrogen bonding interaction with the C1 carbonyl of the thioester, potentially facilitating the hydrolysis. Removal of the PMB protecting group provided us with the seco-acid, which was then subjected to Yamaguchi macrolactonization to provide **1.101**. At this stage, Yam found that the olefin C13-C30 could not be cleaved selectively using ozonolysis or OsO_4 in the presence of the C9 methoxy group and the enoate at C21 of the C-ring. Fortunately, sterically hindered reagents like AD mix-α or β were able to dihydroxylate the C13-C30 olefin of **1.101** selectively. The cleavage of the bond C13-C30 between the vicinal dihydroxyl groups was accomplished by $NaIO_4$ to provide **1.102**. Fuji's chiral BINOL-derived phosphonate **1.68** was used to install the C13-C30 enoate in moderate *Z*:*E* (4:1) selectivity. The

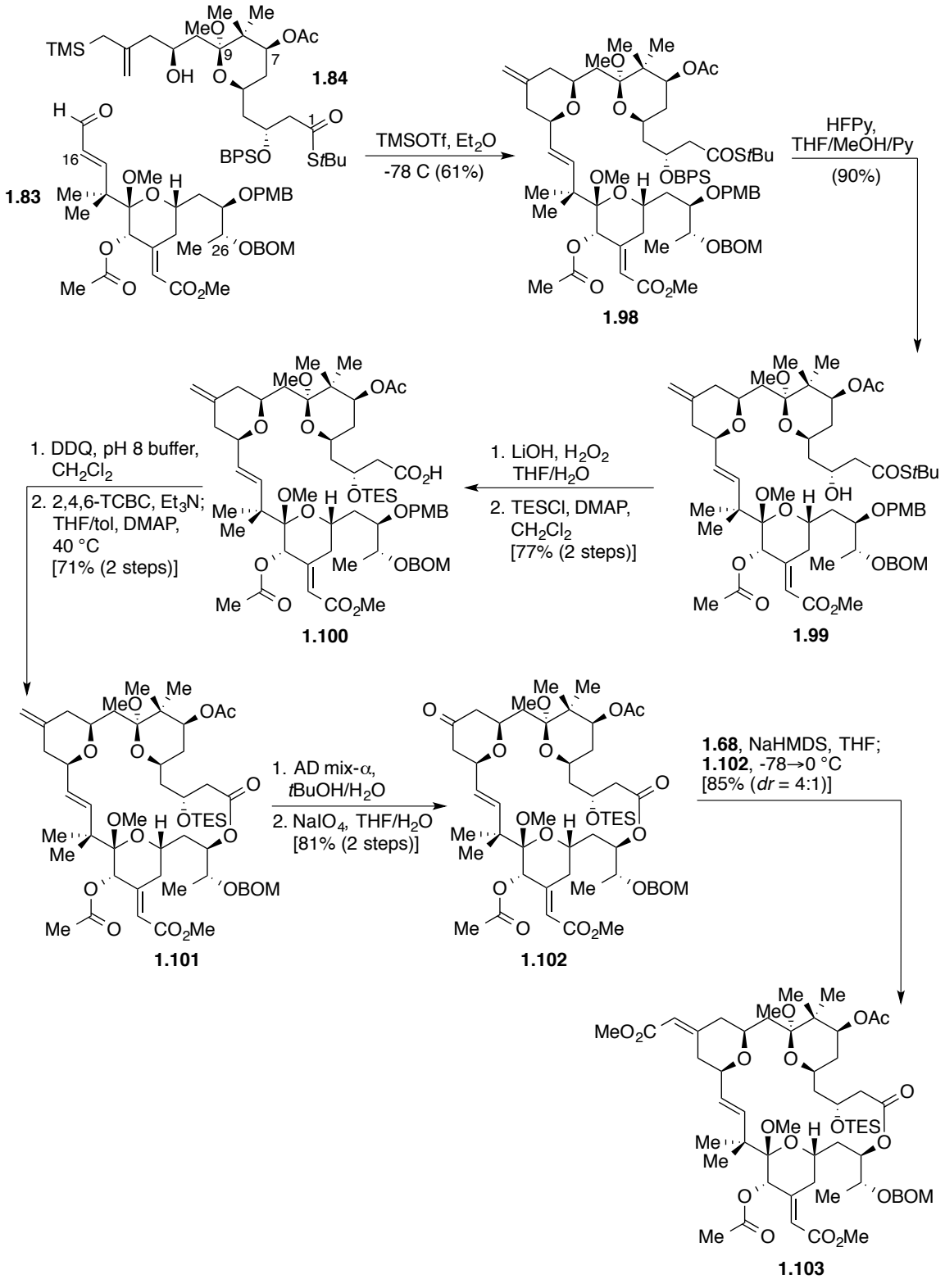


Figure 1.41. Synthesis of the common intermediate **1.103**

intermediate **1.103** was then used to prepare both bryostatins 1 and 7.

When the bisacetate **1.103** was treated with K_2CO_3/MeOH, we were delighted to see that the C20 acetate was selectively cleaved in presence of C7 acetate. The free hydroxy group was immediately esterified with octadienoic anhydride to form the protected bryostatin 1 derivative **1.104**. A global deprotection then completed the first total synthesis of bryostatin 1. From the same intermediate **1.103**, a global deprotection also produced bryostatin 7 in good yield (Figure 1.42).

Biological evaluation of bryostatin 7

The biological evaluation of bryostatin 7 began with finding out the binding affinity toward purified PKCα. The less lipophilic bryostatin 7 was found to bind slightly better (K_i = 0.26 ± 0.06 nM) than bryostatin 1 (K_i = 0.48 ± 0.03 nM) in mouse PKCα. This result is in contrast to that of the other ligands such as phorbol esters and indolactams. To examine any possible PKC isoform selectivity *in vitro*, our collaborators, Dr. Blumberg and coworkers, determined the binding affinities of bryostatin 7 for human PKC isoforms α, βII, δ, and ε (Table 1.3). These isoforms were chosen as PKC βII, δ, and ε are the predominant phorbol ester-sensitive PKC isoforms in the U937 cells and PKC α, δ, and ε are the predominant phorbol ester-sensitive PKC isoforms in the LNCaP cells.[108] Bryostatin 7 showed little selectivity among these PKC isoforms and bound to PKC modestly more strongly than did bryostatin 1. Phorbol ester PDBu also showed little selectivity. Thus for phorbol ester, bryostatin 1, and 7, binding affinities for the different isoforms were very similar, and the binding affinities of them for any particular isozyme were also very similar.

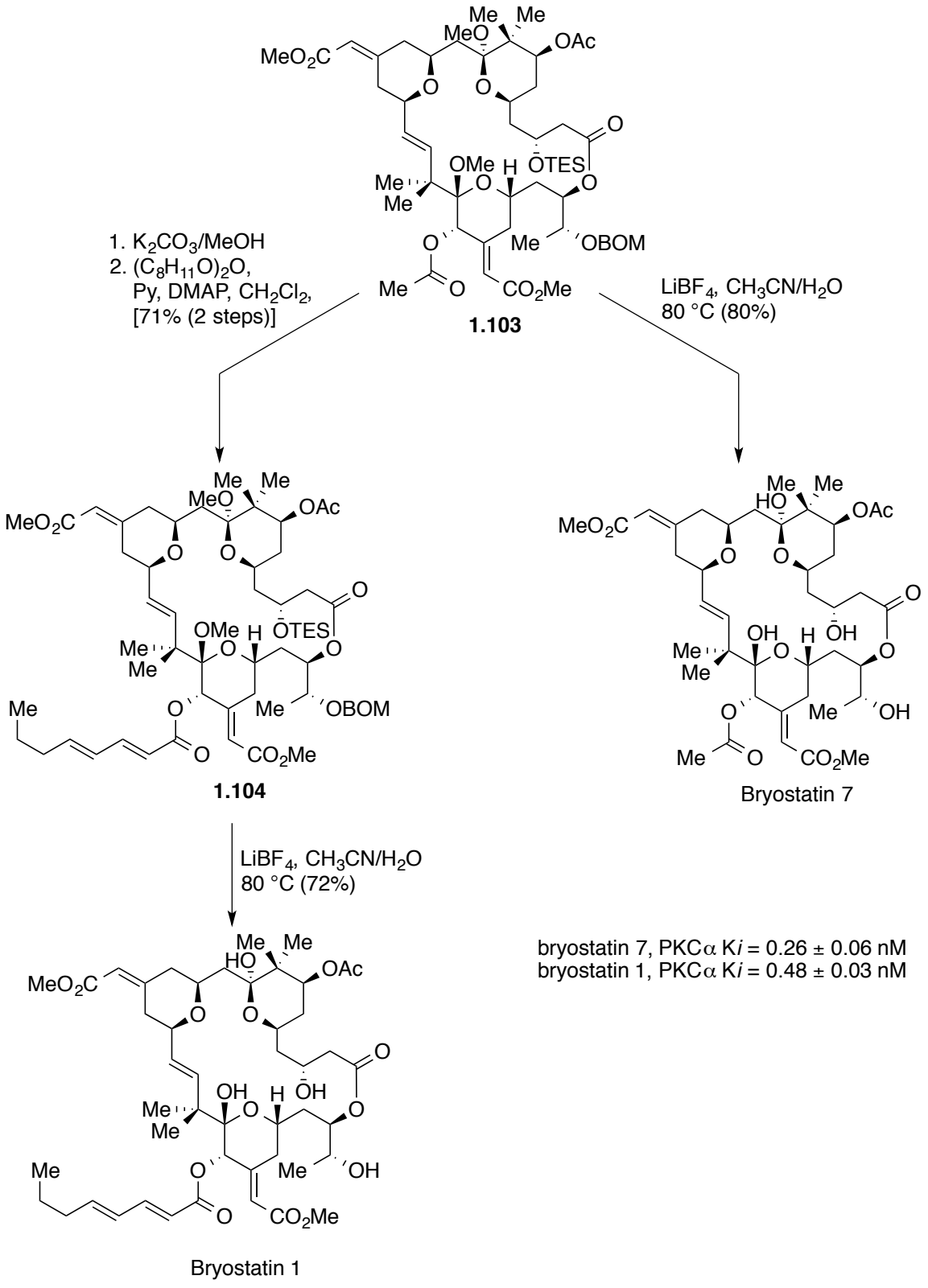


Figure 1.42. Completion of the syntheses of bryostatin 1 and 7

Table 1.3. Comparison of binding affinities (K*i*, nM) to PKC isoforms[127b]

Compounds	Mouse PKCα	Human PKCα	Human PKCβII	Human PKCδ	Human PKCε
PDBu	0.3 ± 0.05	0.28 ± 0.02	0.20 ± 0.0003	0.33 ± 0.08	0.22 ± 0.05
Bryostatin 1	0.48 ± 0.03	0.73 ± 0.05	0.42 ± 0.01	0.26 ± 0.02	0.24 ± 0.01
Bryostatin 7	0.26 ± 0.06	0.44 ± 0.01	0.32 ± 0.01	0.21 ± 0.02	0.16 ± 0.01

[a]Values represent the mean SEM of triplicate independent experiments

As previously described, we examined the biological profile of bryostatin 7 in U937 cells by growth inhibition and cellular attachment assays. Bryostatin 7 closely resembled bryostatin 1 in its effect in U937 cells. Like bryostatin 1, bryostatin 7 caused a very limited, biphasic inhibition of U937 cell proliferation (Figure 1.43). Again, like bryostatin 1, bryostatin 7 was able to suppress the growth inhibition induced by PMA. Bryostatin 7 also caused a reduced level of cell attachment compared to that induced by PMA and, when co-administered, was able to inhibit the attachment induced by PMA. A comparison of the dose-response curves indicated that bryostatin 7 inhibited proliferation slightly more than did bryostatin 1 and induced slightly more attachment than did bryostatin 1. Bryostatin 1 and 7 induced similar levels of TNFα secretion while PMA induced a higher level of secretion. Additionally, bryostatin 7, like bryostatin 1, was able to synergize with Ara-C to induce loss of mitochondrial potential in U937 cells.[127b] Bryostatin 1 and 7 showed similar levels of downregulation of PKC βII, whereas PMA treatment induced less downregulation. Bryostatin 7 showed 3-fold weaker potency than that of bryostatin 1, similar to the potency difference shown in attachment and

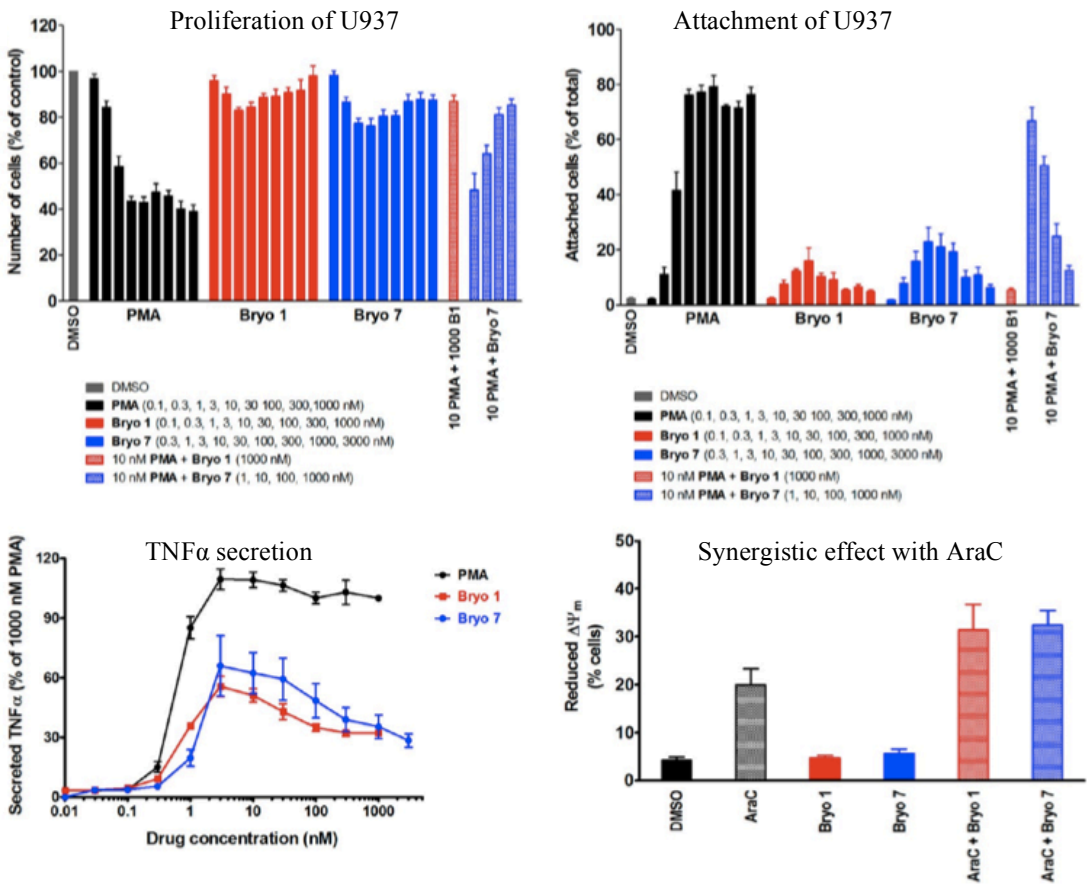


Figure 1.43. Biological responses of U937 leukemia cells to PMA, bryostatin 1, and bryostatin 7

proliferation assays. For PKCδ, bryostatin 1 and 7 showed a biphasic dose-response curve for downregulation, as observed with bryostatin 1 in other cells. Again, bryostatin 7 showed 3-fold weaker potency (Figure 1.44).

In LNCaP cells, bryostatin 7, like bryostatin 1, fails to inhibit proliferation and was able to block the inhibition of proliferation by PMA (Figure 1.45). Similar to bryostatin 1, bryostatin 7 induced minimal secretion of TNFα and inhibits the secretion of TNFα induced by PMA.

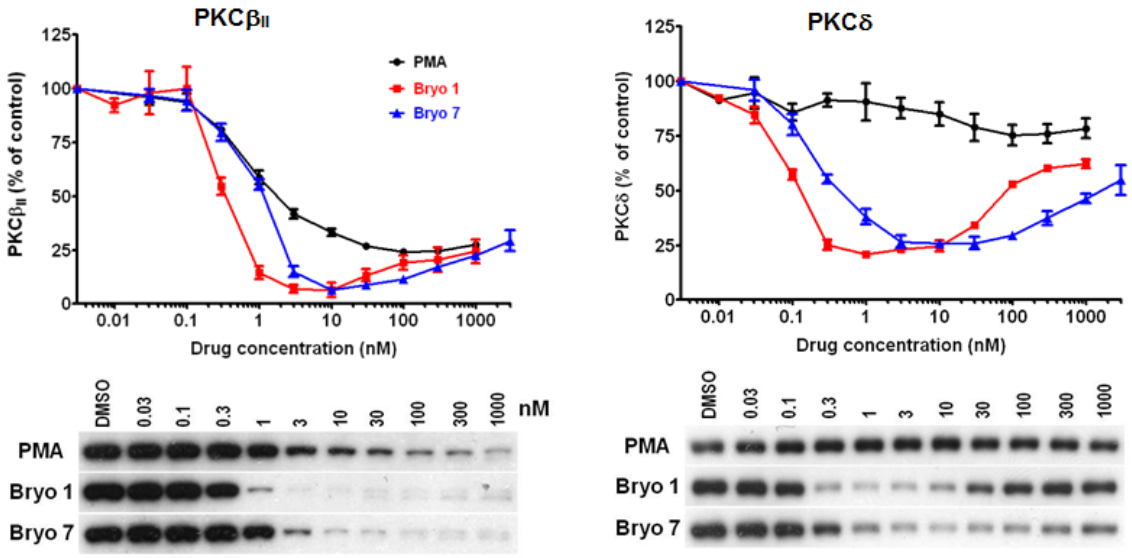


Figure 1.44. Downregulation of PKC βII and PKCδ in U937 cells

Cells were treated with the indicated concentrations of PMA, bryostatin 1, and bryostatin 7 for 24 h. Levels of PKCβII and PKCδ were quantitated in total cell lysates by Simple Western (Simon) using anti-PKCδ antibodies. Loading was normalized to β-actin or α-tubulin, which provided loading controls, and normalized values were expressed relative to that of the DMSO-treated cells. Values represent the mean SEM of three independent experiments. The lower panels show representative images of immunoblots performed on total cell lysates using the same antibodies and visualized by chemiluminescence.

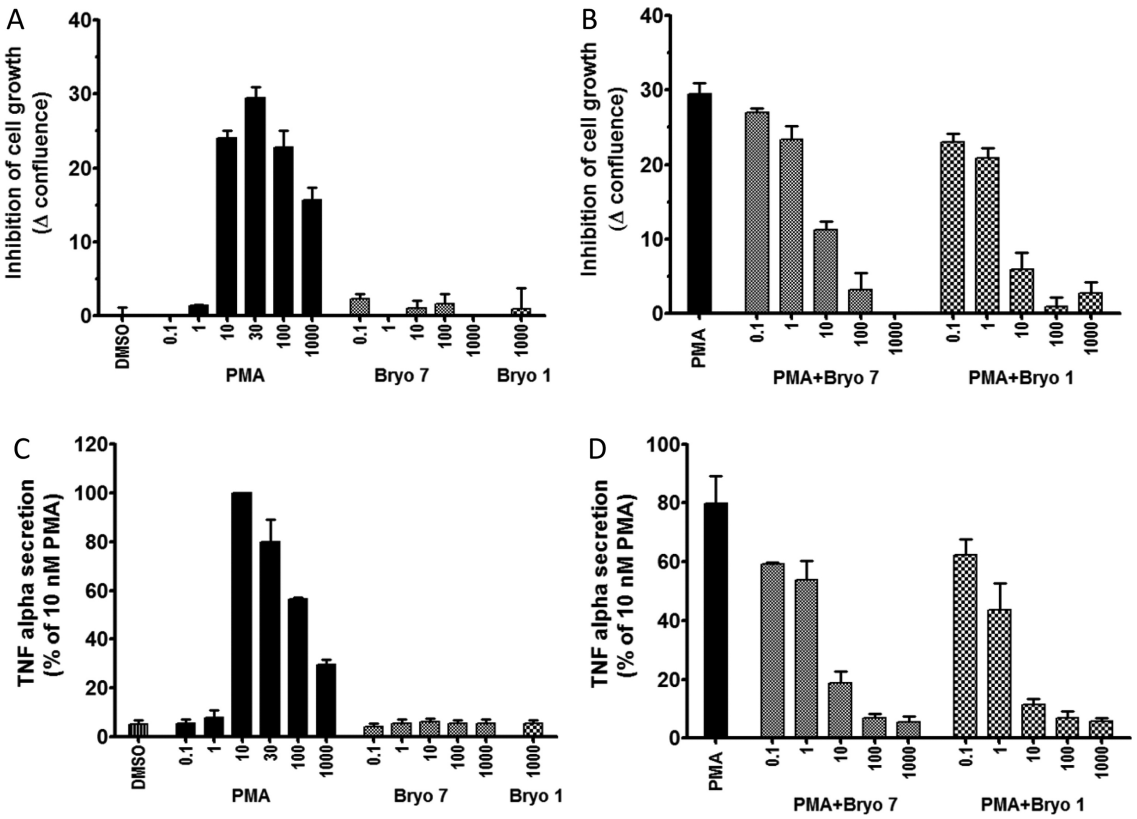


Figure 1.45. Biological response of LNCaP cells to PMA, bryostatin 1, and 7

Previously, it has been mentioned that PMA differed from bryostatin 1 in membrane translocation of PKCδ. Comparison of various phorbol esters and related ligands showed that PKCδ translocation patterns correlated with the tumor-promoting abilities. The compounds that were tumor-promoting showed a similar translocation pattern to that of PMA, whereas the compounds that were nonpromoting showed patterns similar to bryostatin 1. As higher lipophilicity compared to bryostatin 1 was associated with the tumor-promoting ability of PMA, it was hypothesized that bryostatin 7 being less lipophilic than bryostatin 1 should induce even less membrane translocation.

To test this hypothesis, the LNCaP cells were transiently transfected with mouse GFP-PKCδ and the responses to bryostatin 1, 7, and PMA were monitored (Figure 1.46).

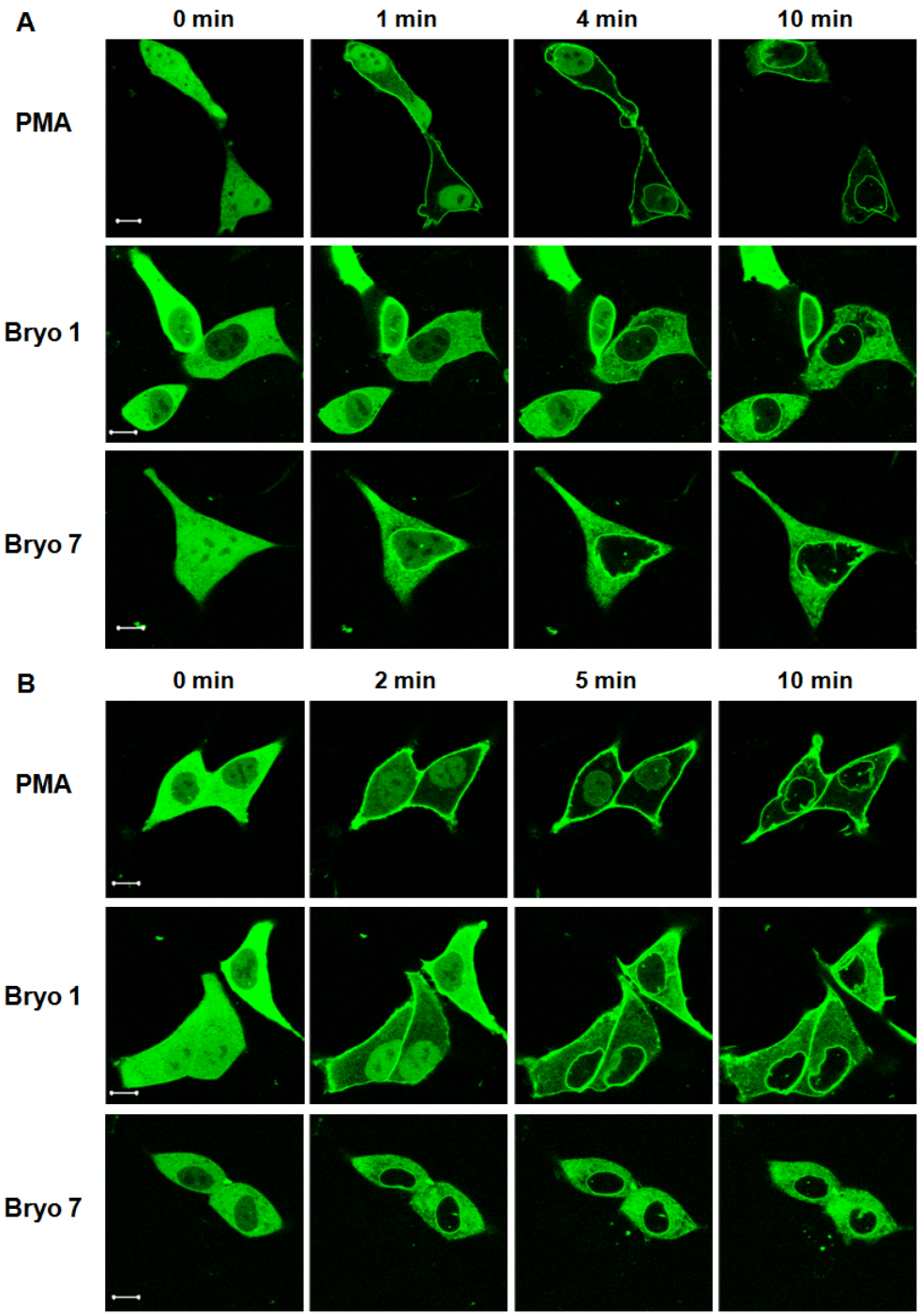


Figure 1.46. Translocation of mouse (A) and human (B) GFP-PKCδ in LNCaP cells after treatment with PMA, bryostatin 1, or bryostatin 7

Bryostatin 7, as expected, caused translocation of mouse GFP-PKCδ to the nuclear and internal membranes, whereas PMA translocated it to the plasma membrane. Interestingly, using human GFP-PKCδ bryostatin 1 initially translocated to plasma membranes although to a much lesser extent than PMA. Bryostatin 7, on the other hand, translocated only to internal membranes. This trend can also be observed in human PKCε for the translocation of YFP-PKCε (Figure 1.47). Clearly in human PKCδ and ε, bryostatin 1 shows some resemblance to PMA while the less lipophilic bryostatin 7 has a distinct pattern. Bryostatin 7 caused no plasma membrane translocation with shift to an internal punctate distribution consistent with association with internal membranes. Additionally, from these studies, it is clear that various ester functionalities at the C20 position play an important role in the translocation behaviors of bryostatins.

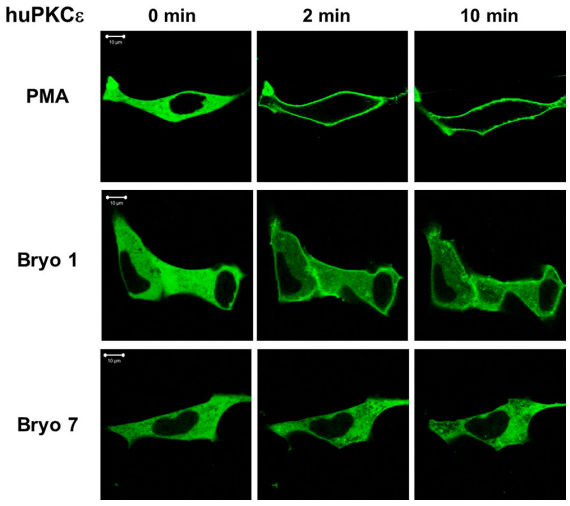


Figure 1.47. Translocation of human YFP-PKCε in LNCaP cells after treatment with PMA, bryostatin 1, and 7.

Conclusions

The power of the pyran annulation has been demonstrated through these complex total syntheses of bryostatin 1, 7, Merle 30, Merle 32, and various other analogues. Our extensive studies on the structure and activity relationship of various functional groups on the northern hemisphere of bryostatin 1 indicated that the C7 acetate, C30 carbomethoxy, C9 hydroxy, C20 octadienoate, and C8 *gem*-dimethyl groups individually do not play critical roles in imparting bryostatin 1 biology. At this point, our understanding is that the subtle balance of the polar and nonpolar functional groups play an important role in the interaction of bryostatin 1 with the lipid bilayer and the various PKC isozymes. This ternary structure of bryostatin 1, PKC, and the lipid bilayer is the key chemical entity that we have to focus on for a better understanding of the unique biological profile of bryostatin 1. To further investigate on the interactions, we endeavored in the syntheses of various analogues, which will be discussed in the Chapter 2.

Experimental section

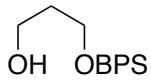
General experimental procedures, materials, and instrumentation

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals*.[131] Diisopropylamine, triethylamine, pyridine, Hünig's base, EtOAc, and CH_2Cl_2 were distilled from CaH_2 under an atmosphere of dry N_2. THF, Et_2O, and toluene were distilled from Na under an atmosphere of dry N_2. $Ti(O\textit{i}Pr)_4$ and $TiCl_4$ were distilled prior to use. A stock solution of $Ti(O\textit{i}Pr)_4$ (1.0 M in CH_2Cl_2) was prepared and used for the BITIP catalyst preparations. The titer of *n*-butyllithium was determined by the method of Eastham and Watson.[132] All other reagents were used without further

purification. Yields were calculated for material judged homogeneous by thin layer chromatography and nuclear magnetic resonance (NMR) spectroscopy. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} plates eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Glassware for reactions was oven dried at 125 ^{o}C and cooled under a dry atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septum-sealed flasks under a nitrogen atmosphere. Column flash chromatography was performed with Silicycle Grade 70 – 230 mesh, 60 – 200 μm, 60 Å silica gel, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Analtech Inc. Silica Gel GF 20 cm × 20 cm × 2000 μm plates or on Merck Kieselgel 60 F_{254} 20 cm × 20 cm × 250 μm plates. Nuclear magnetic resonance spectra were acquired on Varian VXR-500, Varian Inova-500 spectrometer 500 MHz for ^{1}H and 125 MHz for ^{13}C. Prior to use, $CDCl_3$ was filtered through a plug of Fischer Scientific 80 – 200 mesh Alumina Adsorption stored at 110 ^{o}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the signal of residual $CHCl_3$ at 7.27 ppm, or relative to the signal of residual C_6D_5H at 7.16 ppm. Chemical shifts for carbon nuclear magnetic resonance (^{13}C and DEPT) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the center line of the $CDCl_3$ triplet at 77.23 ppm, or relative to the center line of the C_6D_6 triplet at 128.62 ppm. Chemical shifts of the unprotonated carbons (‘C’) for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, bs, d, dd, ddd, dddd, t, td, tt, q, dq, dqd, ddq, ABq,

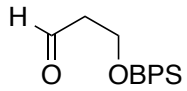
quin, and m stand for the resonance multiplicity singlet, broad singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, doublet of quartet of doublets, doublet of doublet of quartets, AB quartet, quintet, and multiplet, respectively. IR spectra were obtained from a Perkin Elmer FT-IR Paragon 1000 PC spectrometer. Melting points were obtained using a Mel-Temp electrochemical melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin Elmer model 343 polarimeter (Na D line) using a microcell with 1 dm path length. Specific rotations ($[\alpha]^{20}_{D}$, Unit: $^{o}cm^{2}/g$) are based on the equation $\alpha = (100.\ \alpha)/(l.c)$ and are reported as unitless numbers where the concentration c is in g/100 mL and the path length l is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at the University of Utah on a Finnigan MAT 95 double focusing high-resolution mass spectrometer. Compounds were named using ChemBioDraw 13.0.

Experimental procedures and analytical data



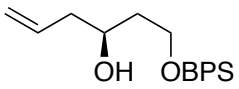
Preparation of 3-((*tert*-butyldiphenylsilyl)oxy)propan-1-ol 1.34.[111a] To a stirring solution of BPSCl (59.0 mL, 227 mmol, 1.00 equiv) in CH_2Cl_2 (1335 mL, 0.17 M) in a 2000 mL round-bottom flask at room temperature were added 1,3-propanediol (81.6 mL, 1.13 mol, 5.00 equiv), triethylamine (63.2 mL, 454 mmol, 2.00 equiv), and DMAP (1.4 g, 11.3 mmol, 0.05 equiv). After 36 h, the mixture was diluted with a 1:1 mixture of EtOAc and hexanes (200 mL) and washed with water (3 × 250 mL) and brine

(100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 7.5 x 28 cm silica gel column eluting with solvent gradient of 10% (1000 mL) through 30% (1000 mL) and 40% EtOAc/hexanes (1000 mL). The eluant was collected in 125 mL fractions and then in two 2000 mL round-bottom flasks. The fractions containing product (27 - 33) and the contents of the two 2000 mL round-bottom flasks were combined and concentrated under reduced pressure to give alcohol **1.34** (58.0 g, 81%) as a crystalline solid: R_f 0.36 (30% EtOAc/hexanes); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.72 – 7.66 (m, 4H), 7.49 – 7.36 (m, 6H), 3.90 – 3.82 (m, 4H), 2.41 (t, *J* = 5.5, 1H), 1.82 (quin, *J* = 5.5 Hz, 2H), 1.07 (S, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 133.4, 130.0, 128.0, 63.6, 62.3, 34.4, 27.0, 19.3.



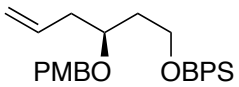
Preparation of 3-((*tert*-butyldiphenylsilyl)oxy)propanal 1.35.[111a] To a stirring solution of oxalyl chloride (26.3 mL, 276 mmol, 1.50 equiv) in CH_2Cl_2 (1842 mL, 0.15 M) in a 3000 mL three-neck round-bottom flask equipped with a mechanical stirrer at -78 ^{o}C was slowly added a solution of dimethyl sulfoxide (39.2 mL, 552 mmol, 3.00 equiv) in CH_2Cl_2 (51.3 mL). After 1 h, a solution of alcohol **1.34** (57.8 g, 184 mmol, 1.00 equiv) in CH_2Cl_2 (92.0 mL) was added via cannula. Triethylamine (128 mL, 920 mmol, 5.00 equiv) was added dropwise via syringe after 1 h. After an additional 1 h, the reaction was determined complete by TLC analysis. The cold bath was removed, the reaction mixture warmed to room temperature, and subsequently quenched with water. The solvent was removed under reduced pressure and then the resulting slurry was diluted with 20% EtOAc/hexanes. The phases were separated and the organic phase was washed with water (3 × 200 mL) and once with brine (200 mL), dried over Na_2SO_4, filtered, and

concentrated under reduced pressure to give pale yellow oil. Purification was accomplished by flash column chromatography on a 7.5 x 12 cm silica gel column eluting with 20% EtOAc/hexanes (6000 mL) and the eluant was collected in 2000 mL fractions. The fractions containing product (2 - 6) were combined and concentrated under reduced pressure to give the aldehyde **1.35** (55.1 g, 96%) as colorless crystalline solid: R_f 0.51 (30% EtOAc/ hexanes); 300 MHz ^{1}H NMR ($CDCl_3$) δ 9.85 (t, *J* = 2.2 Hz, 1H), 7.72 – 7.69 (m, 4H), 7.49 – 7.40 (m, 6H), 4.06 (t, *J* = 6.1 Hz, 2H), 2.64 (td, *J* = 5.9, 2.0 Hz, 2H), 1.09 (s, 9H); 75 MHz ^{13}C NMR ($CDCl_3$) d 202.0, 135.7, 133.4, 130.0, 128.0, 58.4, 46.5, 26.9, 19.3.



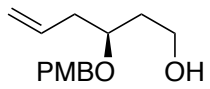
Preparation of (*S*)-1-((*tert*-butyldiphenylsilyl)oxy)hex-5-en-3-ol 1.37.[111a] To a 50 mL three-neck round-bottom flask charged with a magnetic stir bar and equipped with a reflux condenser were added oven-dried 4 Å molecular sieves (1.28 g, 400 g/mol of aldehyde), (*S*)-BINOL (183 mg, 0.640 mmol, 0.20 equiv), CH_2Cl_2 (10 mL, 0.06 M), Ti(O*i*Pr)$_4$ (1.00 M in CH_2Cl_2, 320 μL, 0.320 mmol, 0.10 equiv), and TFA (freshly prepared 0.10 M solution in CH_2Cl_2, 22.4 μL, 0.022 mmol, 7.0 x 10^{-3} equiv). The resulting red-brown solution was heated to 40 ^{o}C for 1 h. The mixture was cooled to room temperature and the reflux condenser exchanged for a rubber septum. A solution of aldehyde **1.35** (1.00 g, 3.20 mmol, 1.00 equiv) in CH_2Cl_2 (1.28 mL) was added via cannula, the mixture stirred for 30 min, and then cooled to -78 ^{o}C. Allyltributyl tin **1.36** (1.29 mL, 4.16 mmol, 1.30 equiv) was added, the mixture stirred for 30 min, and the flask placed in a -20 ^{o}C freezer. After 5 days, the mixture was removed from the freezer and a saturated aqueous $NaHCO_3$ solution (20 mL) was added at 0 ^{o}C. The cold bath was

removed and the mixture stirred for 30 min, allowed to settle, and then filtered through a plug of Celite®. The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic phase was washed with water (2 × 100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2.5 × 24 cm silica gel column eluting with a solvent gradient of hexanes (500 mL) and 5% acetone/ hexanes (750 mL) collecting 25 mL fractions. The fractions containing product (18 - 24) were combined and the solvent was removed under reduced pressure to give the homoallylic alcohol **1.37** (1.12 g, 99%) as colorless oil. The ratio of the enantiomers was determined to be 99:1 (using the other enantiomer made by the same procedure, *er* = 98:2) by HPLC analysis using a Daicel Chiralcel OD-H silica column (length: 25 cm), eluting with a mobile phase of 2.5% 2-propanol/ hexanes and a flow rate of 0.5 mL/ min, respectively, detecting with a Rainin Dynamax Refractive Index Detector Model RI-1: t_r (major) = 8.15 min, t_r (minor) = 9.21 min; R_f 0.58 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.69 – 7.62 (m, 4H), 7.45 – 7.34 (m, 6H), 5.83 (dddd, *J* = 17.2, 10.2, 7.1, 7.1 Hz, 1H), 5.12 – 5.07 (m, 1H), 5.07 – 5.05 (m, 1H), 3.98 – 3.90 (m, 1H), 3.90 – 3.78 (m, 2H), 3.15 (d, *J* = 2.6, 1H), 2.31 – 2.18 (m, 2H), 1.77 – 1.62 (m, 2H), 1.03 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 135.8, 135.2, 133.3, 133.2, 133.0, 130.0, 128.0, 117.6, 71.0, 63.5, 42.2, 38.1, 27.0, 19.2; 125 MHz DEPT ($CDCl_3$) δ CH_3: 27.0, CH_2: 117.6, 63.5, 42.2, 38.1, CH: 135.8, 135.8, 130.0, 130.0, 128.0, 71.0.



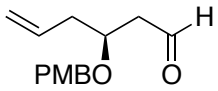
Preparation of (*S*)-*tert*-butyl((3-((4-methoxybenzyl)oxy)hex-5-en-1-yl)oxy)diphenylsilane 1.38.[111a] To a stirring solution of alcohol **1.37** (5.40 g, 15.2 mmol,

1.00 equiv) and freshly prepared 4-methoxybenzyl trichloroacetimidate (10.8 g, 38.1 mmol, 2.50 equiv) in CH_2Cl_2 (31 mL, 0.50 M) in a 100 mL round-bottom flask, under an atmosphere of N_2, was added (±)-camphor-10-sulfonic acid (1.10g, 4.57 mmol, 0.30 equiv) in one portion. The reaction was allowed to proceed for 24 h at rt, after which time TLC analysis indicated essentially complete consumption of the starting material. The reaction mixture was concentrated under reduced pressure, diluted with 20% EtOAc/ hexanes (50 mL), filtered over a pad of Celite®, and concentrated under reduced pressure to give a red slurry. Purification was accomplished by flash column chromatography on a (4.5 x 30) cm silica gel column eluting with 5% EtOAc/ hexanes, collecting 25 mL fractions. The fractions containing product (15 - 27) were combined and concentrated under reduced pressure to give PMB ether **1.38** (5.65 g, 78%) as colorless oil: R_f 0.49 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.66 (m, 4H), 7.46 – 7.36 (m, 6H), 7.23 – 7.19 (m, 2H), 6.86 – 6.83 (m, 2H), 5.85 (dddd, *J* = 17.3, 10.3, 7.0, 7.0 Hz, 1H), 5.12 – 5.05 (m, 2H), 4.45 (ABq, *J* = 11.1 Hz, Δν = 44.9 Hz, 2H), 3.86 – 3.83 (m, 1H), 3.80 (s, 3H), 3.78 – 3.69 (m, 2H), 2.33 (t, *J* = 7.0 Hz, 2H), 1.87 – 1.77 (m, 2H), 1.10 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.3, 135.8, 135.1, 134.2, 134.1, 131.2, 129.8, 129.5, 127.8, 127.8, 117.1, 113.9, 75.3, 71.0, 60.7, 55.5, 38.7, 37.2, 27.1, 19.4; 125 MHz DEPT ($CDCl_3$) δ CH_3: 55.5, 27.1, CH_2: 117.1, 71.0, 60.7, 38.7, 37.2, CH: 135.8, 135.1, 129.8, 129.5, 127.8, 127.8, 113.9, 75.3.



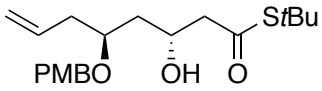
Preparation of (*S*)-3-((4-methoxybenzyl)oxy)hex-5-en-1-ol 1.39.[111a] To a stirring solution of BPS ether **1.38** (32.0 mg, 0.07 mmol, 1.00 equiv) in THF (540 μL, 0.13 M) in a 5 mL round-bottom flask under an atmosphere of N_2, at rt, was added a 1.0

M solution of tetrabutylammonium fluoride (135 μL, 0.13 mmol, 2.00 equiv) in THF, dropwise via syringe. The reaction was allowed to proceed for 15 h at rt, after which time TLC analysis indicated complete consumption of the starting material. The reaction mixture was then quenched with a saturated aqueous NH_4Cl solution (10 mL), extracted with 80% EtOAc/hexanes (3 × 10 mL). The combined organic layer was washed once with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography using a (15 × 2) cm silica gel column, eluting with 30% EtOAc/ hexanes (200 mL), collecting 6 mL fractions. The fractions containing product (29 – 47) were concentrated under reduced pressure to give the primary alcohol **1.39** (15.8 mg, >99%) as colorless oil: R_f 0.10 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.29 – 7.26 (m, 2H), 6.90 – 6.87 (m, 2H), 5.83 (dddd, J = 17.3, 10.2, 7.2, 7.2 Hz, 1H), 5.15 – 5.09 (m, 2H), 4.51 (ABq, J = 11.1 Hz, Δν = 68.9 Hz, 2H), 3.80 (s, 3H), 3.78 – 3.67 (m, 3H), 2.47 – 2.40 (m, 1H), 2.35 (ddd, J = 14.2, 7.0, 7.0 Hz, 1H), 1.81 – 1.71 (m, 2H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.5, 134.5, 130.5, 129.7, 117.7, 114.1, 77.5, 70.9, 61.0, 55.5, 38.2, 36.1; 125 MHz DEPT ($CDCl_3$) δ CH_3: 55.5, CH_2: 117.7, 70.9, 61.0, 38.2, 36.1, CH: 134.5, 129.7, 114.1, 77.5.



Preparation of (*S*)-3-((4-methoxybenzyl)oxy)hex-5-enal 1.25.[111a] To a stirring solution of alcohol **1.39** (1.75 g, 7.40 mmol, 1.00 equiv) in CH_2Cl_2 (80 mL, 0.10 M) in a 250 mL round-bottom flask under an atmosphere of N_2, at -10 ^{o}C, was added N,N-diisopropylethylamine (9.75 g, 52.0 mmol, 7.00 equiv). After 10 min at -10 ^{o}C, dimethyl sulfoxide (5.7 mL, 74.0 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for an additional 10 min. Sulfur trioxide pyridine

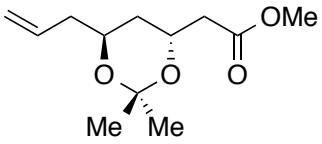
complex (5.10 g, 29.6 mmol, 4.00 equiv) was then added in one portion. The reaction was allowed to proceed for 1 h at -10 ^{o}C, after which time TLC analysis indicated complete consumption of the starting material. The reaction was quenched by adding saturated aqueous solution of $NaHCO_3$ (100 mL) and the phases were separated. The aqueous phase was extracted with 20% EtOAc/ hexanes (3 × 50 mL). The combined organic phase was washed twice with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was by flash column chromatography on a (15 × 4) cm silica gel column eluting with 10% EtOAc/ hexanes (750 mL), collecting 10 mL fractions. The fractions containing product (29 – 45) were concentrated under reduced pressure to give the aldehyde **1.25** (1.60 g, 92%) as colorless oil: R_f 0.60 (50% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 9.77 (dd, *J* = 2.3, 1.8 Hz, 1H), 7.26 – 7.23 (m, 2H), 6.90 – 6.86 (m, 2H), 5.81 (dddd, *J* = 14.3, 9.7, 7.1, 7.1 Hz, 1H), 5.16 – 5.14 (m, 1H), 5.12 (m, 1H), 4.51 (ABq, *J* = 11.1 Hz, Δν = 40.5 Hz, 2H), 4.05 – 4.01 (m, 1H), 3.81 (s, 3H), 2.67 (ddd, *J* = 14.3, 7.1, 2.3 Hz, 1H), 2.56 (ddd, *J* = 16.7, 4.4, 1.8 HZ, 1H), 2.47 – 2.42 (m, 1H), 2.40 – 2.35 (m, 1H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.6, 159.5, 133.8, 130.3, 129.6, 118.4, 114.0, 73.5, 71.1, 55.5, 48.2, 38.5; 125 MHz DEPT ($CDCl_3$) δ CH_3: 55.5, CH_2: 118.4, 71.1, 48.2, 38.5, CH: 201.6, 133.8, 129.6, 114.0, 73.5.



Preparation of *S*-(*tert*-butyl) (3*R*,5*S*)-3-hydroxy-5-((4-methoxybenzyl)oxy)oct-7-enethioate 1.41.[111a] To a stirring solution of aldehyde **1.25** (103 mg, 0.44 mmol, 1.00 equiv) in toluene (3.0 mL, 0.15 M) in a 10 mL round-bottom flask, under an atmosphere of N_2, at -78 ^{o}C, was added a freshly prepared 1.0 M solution of $TiCl_2(OiPr)_2$ in toluene (1.10 mL, 1.1 mmol, 2.50 equiv) dropwise via syringe directly into the reaction mixture.

The resulting bright yellow solution was allowed to stir for 15 min, followed by dropwise addition of thioketene acetal **1.26** (233 mg, 1.14 mmol, 2.60 equiv), in toluene (300 μL), down the inside of the reaction flask over a 5-min period. An additional 200 μL of toluene rinse was used to transfer the remaining thioketene acetal residue from the syringe into the reaction flask. TLC analysis after 4 h, at -78 ^{o}C, indicated complete consumption of the aldehyde starting material. The reaction was quenched by transferring directly into a 250 mL Erlenmeyer flask that contained a vigorously stirring mixture of CH_2Cl_2 (100 mL) and aqueous pH 7.0 phosphate buffer (50 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with a saturated NH_4Cl solution (2 × 50 mL), then with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give pale yellow oil. Purification was accomplished by flash column chromatography on a (3.5 × 20) cm silica gel column eluting with a solvent gradient of hexanes (50 mL) through 10% EtOAc/ hexanes (250 mL) and 20% EtOAc/ hexanes (200 mL), collecting 10 mL fractions. The fractions containing product (50 – 62) were combined and concentrated under reduced pressure to give β-hydroxy thioester **1.41** (154.7 mg, 96%) as colorless oil: R_f 0.67 (50% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.29 – 7.26 (m, 2H), 6.90 – 6.87 (m, 2H), 5.81 (dddd, *J* = 17.2, 10.2, 7.1, 7.1 Hz, 1H), 5.14 – 5.07 (m, 2H), 4.51 (ABq, *J* = 11.0 Hz, Δν = 57.0 Hz, 2H), 4.33 – 4.26 (m, 1H), 3.81 (s, 3 H), 3.79 – 3.76 (m, 1H), 3.17 (d, *J* = 3.8 Hz, 1H), 2.64 – 2.57 (m, 2H), 2.45 – 2.40 (m, 1H), 2.34 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 1.68 – 1.57 (m, 2H), 1.47 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 200.1, 159.5, 134.5, 130.6, 129.8, 117.7, 114.2, 114.1, 75.5, 71.3, 66.0, 55.5, 51.6, 48.6, 40.2, 38.4, 30.0; 125 MHz DEPT ($CDCl_3$) δ CH_3: 55.5, 30.0, CH_2:

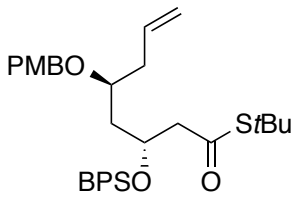
117.7, 71.3, 51.6, 40.2, 38.4, CH: 134.5, 129.8, 114.2, 114.1, 75.5, 66.0.



Preparation of methyl 2-((4*R*,6*S*)-6-allyl-2,2-dimethyl-1,3-dioxan-4-yl)acetate 1.42. To a stirring solution of a thioester **1.41** (200 mg, 0.545 mmol, 1.00 equiv) in CH_2Cl_2 (2.7 mL, 0.20 M) in a 15 mL round-bottom flask under an atmosphere of N_2, was added TBSCl (205 mg, 1.36 mmol, 2.50 equiv) and imidazole (92.6 mg, 1.36 mmol, 2.50 equiv) and the mixture was allowed to stir for 24 h. The reaction was judged complete by TLC analysis. The phases were separated and the aqueous phase was extracted with 20% EtOAc/ hexanes (3 × 20 mL). The combined organic phases were washed once with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure and used for the next step without further purification.

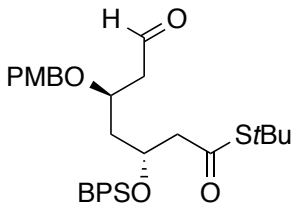
To a stirring crude solution of a thioester **1.41** (assumed to be 0.545 mmol, 1.00 equiv), CH_2Cl_2 (9.1 mL, 0.06 M), and water (1.6 mL) in a 15 mL round-bottom flask under an atmosphere of N_2, at rt, was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (185.6 mg, 0.817 mmol, 1.50 equiv) in one portion. The reaction was allowed to proceed for 1 h, during which time solution color changed from dark green to orange. TLC analysis at 1 h indicated complete consumption of PMB ether starting material. The reaction mixture was diluted with 30% EtOAc/ hexanes (50 mL) and filtered, over a plug of Celite®, Florisil®, and $MgSO_4$. The filtrate was concentrated under reduced pressure to give a mixture of anisaldehyde and mono deprotected product, which was taken directly to the next reaction without further purification.

To a stirring solution of crude silyl ether (assumed to be 0.545 mmol, 1.00 equiv) and MeOH (4 mL) in a 15 mL round-bottom flask under N_2, at rt, was added p-toluenesulfonic acid monohydrate (10.3 mg, 0.05 mmol, 0.10 equiv) in one portion. TLC analysis after 30 min shows complete consumption of TBS ether starting material. The reaction mixture was diluted with 2,2-dimethoxypropane (4 mL) and stirred for an additional 10 min. The magnetic stir bar was extracted and the solvent was removed under reduced pressure. The concentrate was diluted with 2,2-dimethoxypropane (2 mL) and the mixture was again concentrated under reduced pressure. 2,2-dimethoxypropane (4 mL) was added to the concentrated mixture and the reaction was allowed to proceed for 15 min, after which time TLC analysis indicated complete consumption of diol. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with a saturated aqueous $NaHCO_3$ solution (10 mL), brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product as red oil. Purification was done by flash column chromatography on a (2.5 x 10) cm column, eluting with 5% EtOAc/ hexanes, collecting 10 mL fractions. The fractions containing product (14 - 17) were combined and concentrated under reduced pressure to give acetonide **1.42** (74 mg, 25%) as colorless oil: R_f 0.50 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 5.80 (dddd, *J* = 17.2, 10.2, 6.8 Hz, 1H), 5.13 – 5.04 (m, 2H), 4.27 (dddd, *J* = 11.4, 9.5, 8.2, 5.5 Hz, 1H), 3.87 (dddd, *J* = 15.3, 12.9, 9.0, 6.2 Hz, 1H), 3.69 (s, 1H), 2.55 (dd, *J* = 15.6, 8.1 Hz, 1H), 2.44 (dd, *J* = 15.6, 5.4 Hz, 1H), 2.32 (ddd, *J* = 14.2, 6.0, 6.0 Hz, 1H), 2.21 (ddd, *J* = 14.4, 7.2, 6.0, 1H), 1.72 (ddd, *J* = 12.8, 9.1, 6.0, 1H), 1.63 (ddd, *J* = 12.9, 10.0, 6.0, 1H), 1.37 (s, 3H), 1.34 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.6, 134.5, 117.2, 100.8, 66.2, 63.6, 51.8, 40.8, 40.2, 37.5, 24.9, 24.8.



Preparation of *S*-(*tert*-butyl) (3*R*,5*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-5-((4-methoxybenzyl)oxy)oct-7-enethioate 1.43.[111a] To a stirring solution of a β-hydroxy thioester **1.41** (1.38 g, 3.78 mmol, 1.00 equiv) and imidazole (773 mg, 11.4 mmol, 3.00 equiv) in DMF (13.0 mL, 0.30 M) in a 25 mL round-bottom flask under an atmosphere of N_2, at rt, was added *tert*-butyl(chloro)diphenylsilane (1.18 mL, 4.54 mmol, 1.20 equiv) via syringe. The mixture was allowed to stir for 24 h, after which the reaction was quenched with addition of saturated aqueous solution of $NaHCO_3$. The phases were separated and the aqueous phase was extracted with 20% EtOAc/ hexanes (3 × 50 mL). The combined organic phase was washed once with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a silica gel column (2.5 × 30 cm) eluting with 5% EtOAc/ hexanes, collecting 10 mL fractions. The fractions containing product (25 - 35) were combined and concentrated under reduced pressure to give silyl ether **1.43** (2.10 g, 92%) as pale yellow oil: R_f 0.47 (30%EtOAc/hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.74 – 7.70 (m, 4H), 7.45 – 7.36 (m, 6H), 7.14 – 7.11 (m, 2H), 6.86 – 6.83 (m, 2H), 5.61 (dddd, *J* = 17.4, 10.2, 7.1, 7.1 Hz, 1 H), 4.99 – 4.95 (m, 2H), 4.41 (tt, *J* = 6.6, 5.6 Hz, 1H), 4.31 (d, *J* = 11.1 Hz, 1H), 4.06 (d, *J* = 11.1 Hz, 1H), 3.81 (s, 3 H), 3.38 – 3.34 (m, 1H), 2.72 (dd, *J* = 14.6, 6.2 Hz, 1H), 2.66 (dd, *J* = 14.6, 5.8 Hz, 1H), 2.14 – 2.05 (m, 2H), 1.76 (ddd, *J* = 14.2, 8.2, 5.9 Hz, 1H), 1.66 (ddd, *J* = 14.2, 6.2, 3.9 Hz, 1H), 1.44 (s, 9H), 1.04 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 159.1, 136.2, 136.1, 134.5, 134.0, 131.0,

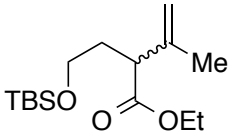
129.9, 129.8, 129.3, 127.8, 127.8, 117.3, 113.8, 75.7, 70.3, 69.2, 55.4, 53.1, 48.1, 42.4, 38.6, 30.0, 27.2, 19.5.



Preparation of *S*-(*tert*-butyl) (3*R*,5*R*)-3-((*tert*-butyldiphenylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-7-oxoheptanethioate 1.23.[111a] To a stirring solution of olefin **1.43** (1.91 g, 3.16 mmol, 1.00 equiv) in THF (14 mL, 0.22 M), *t*-butanol (14 mL), and water (3 mL) in a 50 mL round-bottom flask under N_2, at rt, was added 4-methylmorpholine N-oxide (462 mg, 3.94 mmol, 1.25 equiv) in one portion. A 0.10 M solution of OsO_4 (1.6 mL, 0.157 mmol, 0.05 equiv) in THF was added dropwise, via syringe. The reaction was allowed to proceed for 14 h, after which time TLC analysis indicated complete consumption of olefin starting material. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution in one portion. The mixture was stirred for 1 h, during which time a color change from yellow to dark brown was observed. The quenched reaction mixture was diluted with water (20 mL), EtOAc (100 mL), and the phases were separated. The aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic phases were washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude diol as thick yellow liquid, which was then taken directly to the next reaction without any further purification.

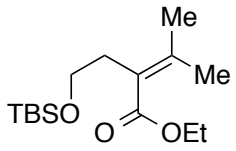
To a stirring solution of crude diol (assumed to be 3.16 mmol, 1.00 equiv) in benzene (32 mL, 0.10 M) under an atmosphere of N_2, at rt, was added $Pb(OAc)_4$ (1.54 g, 3.47 mmol, 1.10 equiv) in three portions over 10 min. After 1 h, TLC analysis indicated

complete consumption of the starting material. The reaction mixture was diluted with hexanes (100 mL) then filtered over a pad of Celite® and Na_2SO_4. The filtrate was concentrated under reduced pressure then high vacuum to yield aldehyde **1.23** (2.00 g, quant. over 2 steps) as viscous colorless oil: R_f = 0.72 (50% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 9.54 (t, *J* = 2.3 Hz, 1H), 7.73 – 7.69 (m, 4H), 7.46 – 7.37 (m, 6H), 7.11 – 7.08 (m, 2H), 6.85 – 6.82 (m, 2H), 4.43 (tt, *J* = 6.4, 5.9 Hz, 1H), 4.19 (ABq, *J* = 11.0 Hz, Δν = 40.0 Hz, 2H), 3.85 – 3.80 (m, 2H), 3.80 (s, 3H), 2.72 (dd, *J* = 14.6, 5.9 Hz, 1H), 2.63 (dd, *J* = 14.6, 6.4 Hz, 1H), 2.34 (ddd, *J* = 16.5, 6.6, 2.3 Hz, 1H), 2.26 (ddd, *J* = 16.5, 4.9, 2.3 Hz, 1H), 1.96 (ddd, *J* = 14.3, 6.8, 5.9 Hz, 1H), 1.64 (ddd, *J* = 14.3, 5.9, 5.5 Hz, 1H), 1.43 (s, 9H), 1.05 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.2, 197.7, 159.4, 136.1, 136.1, 133.9, 133.7, 130.3, 130.0, 130.0, 129.5, 127.9, 127.9, 113.9, 71.4, 70.7, 68.5, 55.5, 52.6, 48.6, 48.3, 42.8, 29.9, 27.1, 19.6.



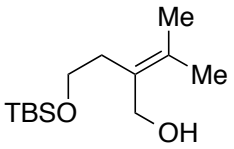
Preparation of ethyl 2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-methylbut-3-enoate 1.44.[111a] To a cooled (0 °C) stirring solution of diisopropylamine (19.7 mL, 140 mmol, 1.2 equiv) in THF (702 mL, 0.20 M) in a flame-dried 2000 mL round-bottom flask under an atmosphere of N_2 was added a 2.47 M solution of *n*BuLi (52.1 mL, 130 mmol, 1.10 equiv) in hexanes, dropwise via syringe. The resulting yellow solution was stirred for 30 min at 0 °C and then cooled to -78 °C. Ethyl 3,3-dimethylacrylate **1.24** (16.3 mL, 117 mmol, 1.00 equiv) was added dropwise via syringe to the reaction mixture. After 30 min, a solution of 2-iodo-1-(*tert*-butyldimethylsilanoxy)-ethane (33.5 g, 117 mmol, 1.00 equiv) in THF (17 mL) was added dropwise via cannula to the reaction mixture. An

additional THF rinse (5 mL) was used to transfer the remaining iodide residue into the reaction flask via cannula. The reaction was allowed to proceed for 15 h, allowing the -78 ^{o}C bath to expire overnight, after which the TLC analysis indicated complete consumption of the ester starting material. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (250 mL). The resulting mixture was diluted with water (250 mL) and Et_2O (500 mL) and the phases were separated. The aqueous phase was extracted with Et_2O (2 × 250 mL). The combined organic phases were washed with brine (250 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product as red oil. Purification was accomplished by flash column chromatography on a (5 x 30) cm silica gel column eluting with a solvent gradient of hexanes (2000 mL) through 1% EtOAc/ hexanes (2000 mL), collecting 25 mL fractions. The fractions containing product (8 - 100) were combined and concentrated under reduced pressure to give alkylated ester **1.44** (26.8 g, 80%) as colorless oil: R_f 0.58 (15% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 4.88 (quin, *J* = 1.5 Hz, 1H), 4.87 – 4.86 (m, 1H), 4.12 (app dq, *J* = 7.0, 1.1 Hz, 2H), 3.58 (app t, *J* = 6.5 Hz, 2H), 3.23 (t, *J* = 7.7 Hz, 1H), 2.06 (dddd, *J* = 13.9, 8.1, 6.2, 6.2 Hz, 2H), 1.78 – 1.71 (m, 1H), 1.73 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 0.88 (s, 9H), 0.02 (s, 3H), 0.02 (s, 3H).



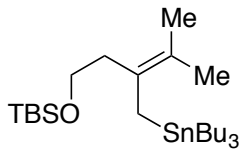
Preparation of ethyl 2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-methylbut-2-enoate 1.45.[111a] An oven-dried 1000 mL round-bottom flask was charged with ester **1.44** (11.2 g, 39.1 mmol, 1.00 equiv) and a magnetic stir bar. The flask was purged by a steady stream of N_2 for 15 min. THF (391 mL, 0.10 M), drawn from a 500 mL round-bottom

flask that was purged with N_2 for 1 h, was added to the reaction flask via a Gastight® syringe. After cooling the mixture to 0 °C, KO*t*Bu (4.39 g, 39.1 mmol, 1.00 equiv) was added to the reaction flask in one portion, under a steady steam of N_2. The reaction was allowed to proceed for 3 h at 0 °C and then quenched by transfer into a 1000 mL Erlenmeyer flask that contained a stirring mixture of a saturated aqueous NH_4Cl solution (100 mL) and Et_2O (100 mL). The phases were separated. The organic phase was washed twice with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give pale yellow oil. Purification was accomplished by flash column chromatography on a (5 x 25) cm silica gel column eluting with 5% EtOAc/ hexanes (1000 mL), collecting 25 mL fractions. The fractions containing product (22 - 56) were combined and concentrated under reduced pressure to give the ester **1.45** (9.90 g, 95%) as colorless oil: R_f 0.34 (10% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 4.19 (q, *J* = 7.1 Hz, 2H), 3.64 (t, *J* = 7.2 Hz, 2H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.00 (s, 3H), 1.86 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 169.6, 145.3, 124.4, 62.5, 60.2, 33.7, 26.2, 23.3, 22.6, 18.6, 14.5, -5.1.



Preparation of 2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-3-methylbut-2-en-1-ol 1.46.[111a] To a stirring solution of ester **1.45** (6.59 g, 23.0 mmol, 1.00 equiv) and CH_2Cl_2 (230 mL, 0.10 M) in a 500 mL round-bottom flask under an atmosphere of N_2, at -20 °C, was added a 1.0 M solution of diisobutylaluminum hydride (57.5 mL, 57.5 mmol, 2.5 equiv) in CH_2Cl_2, dropwise, via syringe. The reaction was allowed to proceed for 2 h at -20 °C, after which time the reaction was judged complete by TLC analysis. The reaction

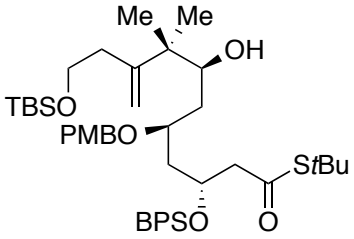
was quenched by dropwise addition of methanol. The mixture was transferred into a 1000 mL Erlenmeyer flask containing a vigorously stirring mixture of a saturated aqueous solution of potassium sodium tartrate (200 mL) and CH_2Cl_2 (200 mL). The resulting mixture stirred for overnight, after which the phases were separated and the aqueous phase extracted with CH_2Cl_2 (3 × 100 mL). The organic phases were combined and washed twice with brine (100 mL), dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to give pale yellow oil. Purification was accomplished by flash column chromatography on a 3 × 25 cm silica gel column eluting with 2.5% EtOAc/ hexanes (2000 mL), collecting in 25 mL fractions. The fractions containing product (25 - 65) were combined and concentrated under reduced pressure to give the alcohol **1.46** (4.6 g, 88%) as clear colorless oil: R_f 0.09 (15% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 4.10 (s, 2 H), 3.70 (t, J = 6.0 Hz, 2H), 3.25 (s, 1H), 2.42 (t, J = 5.7 Hz, 2H), 1.76 (s, 3H), 1.70 (s, 3H), 0.91 (s, 9H), 0.08 (s, 3H).



Preparation of *tert*-butyldimethyl((4-methyl-3-((tributylstannyl)methyl)pent-3-en-1-yl)oxy)silane 1.22.[111a] To a stirring solution of allylic alcohol **1.46** (4.59 g, 18.8 mmol, 1.00 equiv) in THF (19 mL, 1.00 M) in a flame-dried 250 mL round-bottom flask under an atmosphere of N_2, at -78 °C, was added a 2.53 M solution of *n*BuLi (8.17 mL, 20.7 mmol, 1.10 equiv) in hexanes dropwise via syringe. The resulting yellow solution was stirred for 30 min at -78 °C. Simultaneously, to a stirring solution of diisopropylamine (2.76 mL, 19.7 mmol, 1.05 equiv) in THF (20 mL, 1 M) in a 100 mL round-bottom flask under an atmosphere of N_2, at 0 °C, was added a 2.53 M solution of

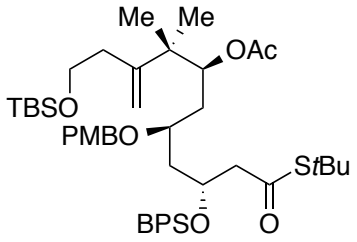
*n*BuLi (7.72 mL, 19.5 mmol, 1.04 equiv) in hexanes dropwise via syringe. After 45 min, freshly prepared tributyltin hydride (5.2 mL, 19.3 mmol, 1.03 equiv) was added via syringe to the freshly prepared LDA solution. At the same time, methanesulfonyl chloride (1.45 mL, 18.8 mmol, 1.00 equiv) was added to the lithium alkoxide solution at -78 °C. After 1.5 h, the Bu_3SnLi solution was added dropwise to the reaction flask via cannula. An additional THF (2 mL) rinse was used to transfer Bu_3SnLi residue from the flask into the reaction mixture via cannula. The reaction was allowed to proceed for 2 h at -78 °C, then for an additional 18 h during which time the -78 °C bath was allowed to expire. The mixture was quenched by addition of water (25 mL), and then diluted with 10% EtOAc/ hexanes (200 mL) and the phases were separated. The organic phases were washed twice with water (50 mL), once with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give yellow oil. Purification was accomplished by flash column chromatography on a 3 x 30 cm silica gel column eluting with hexanes (2000 mL), collecting in 8 mL fractions. The fractions containing product (20 – 133) were combined and concentrated under reduced pressure to give allylstannane **1.22** (3.30 g, 78%) as colorless oil: R_f 0.17 (hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 3.63 (t, J = 7.7 Hz, 2H), 2.20 (t, J = 7.7 Hz, 2H), 1.72 (app s, flanked by Sn satellites, 1H), 1.67 (app s, flanked by Sn satellites, 3H), 1.59 (app s, flanked by Sn satellites, 3H), 1.56 – 1.40 (m, 6H), 1.31 (sextet, J = 7.3 Hz, 6 H), 0.92 (s, 9H), 0.90 (t, J = 7.3 Hz, 9H), 0.85 – 0.82 (m, 6H), 0.08 (s, 3H); 125 MHz 13C NMR (CDCl3) δ 128.3 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 23 Hz), 120.6 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 22 Hz), 62.6 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 7 Hz), 38.7, 29.4 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 10 Hz), 27.7 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 27 Hz), 26.3, 21.1 (flanked by Sn satellites, $J_{C\text{-}Sn}$ = 6 Hz), 20.4 (flanked by Sn

satellites, $J_{C\text{-}Sn}$ = 6 Hz), 18.7, 17.2 (flanked by Sn satellite doublets, $J_{C\text{-}Sn}$ = 124 Hz), 13.9, 10.1 (flanked by Sn satellite doublets, $J_{C\text{-}Sn}$ = 153 Hz), - 5.0 (flanked by Sn satellite doublets, $J_{C\text{-}Sn}$ = 28 Hz).



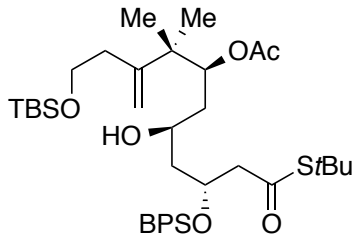
Preparation of *S*-(*tert*-butyl) (3*R*,5*S*,7*S*)-11-((*tert*-butyldimethylsilyl)oxy)-3-((*tert*-butyldiphenylsilyl)oxy)-7-hydroxy-5-((4-methoxybenzyl)oxy)-8,8-dimethyl-9-methyleneundecanethioate 1.48.[111a] To a stirring solution of aldehyde **1.23** (2.00 g, 3.29 mmol, 1.00 equiv) in toluene (33 mL, 0.10 M) in a 100 mL round-bottom flask under an atmosphere of N_2, at -78 °C, was added a freshly prepared 3.0 M solution of Me_2AlCl (5.5 mL, 16.5 mmol, 5.00 equiv) in toluene dropwise, via syringe, down the inside of the flask. The solution was stirred for 10 min at -78 °C, then stannane **1.22** (2.20 g, 4.28 mmol, 1.30 equiv) in toluene (3.5 mL) was added dropwise, via syringe, down the inside of the reaction flask. The reaction was allowed to proceed for 2 h at -78 °C, and then quenched by direct transfer into a 500 mL Erlenmeyer flask that contained a vigorously stirring mixture of a saturated aqueous potassium sodium tartrate solution (100 mL) and 20% EtOAc/hexanes (100 mL). The resulting mixture was allowed to stir for overnight and then the phases were separated. The aqueous phase was extracted with 30% EtOAc/hexanes (3 × 100 mL). The combined organic phases were washed once with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product as pale yellow oil. Purification was accomplished by flash column chromatography on a (2.5 × 22) cm silica gel column, eluting with a solvent gradient of

5% EtOAc/hexanes (500 mL) through 10% EtOAc/hexanes (750 mL), collecting 10 mL fractions. The fractions containing product (59 – 103) were combined and concentrated under reduced pressure to give the alcohol **1.48** (2.1 g, 72%) as a single diastereomer by ^{1}H and ^{13}C NMR, and as colorless oil: R_f 0.64 (30% EtOAc/hexanes); 400 MHz 1H NMR ($CDCl_3$) δ 7.76 – 7.72 (m, 4H), 7.46 – 7.38 (m, 6H), 7.16 – 7.14 (m, 2H), 6.86 – 6.83 (m, 2H), 4.99 (s, 1H), 4.93 (s, 1H), 4.34 (app quin, *J* = 5.6 Hz, 1H), 4.24 (ABq, *J* = 11.0 Hz, Δν = 49.0 Hz, 2H), 3.81 – 3.77 (m, 2H), 3.81 (s, 3H), 3.68 (d, *J* = 9.3 Hz, 1H), 3.64 – 3.59 (m, 1H), 2.72 (dd, *J* =14.3, 5.6 Hz, 1H), 2.66 (dd, *J* = 14.3, 5.6 Hz, 1H), 2.43 (d, *J* = 2.6 Hz, 1H), 2.28 (ddd, *J* = 15.0, 7.3, 7.3 Hz, 1H), 2.23 (ddd, *J* = 15.0, 6.2, 6.2 Hz, 1H), 1.98 (td, *J* = 13.9, 6.6 Hz, 1H), 1.60 (td, *J* = 13.9, 5.6 Hz, 1H), 1.46 (s, 9H), 1.26 – 1.16 (m, 2H), 1.06 (s, 9H), 0.97 (s, 3H), 0.93 (s, 9H), 0.90 (s, 3H), 0.09 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.8, 159.2, 151.8, 136.2, 136.1, 134.1, 133.9, 130.9, 129.9, 129.8, 129.5, 127.8, 113.8, 111.2, 74.4, 71.6, 71.0, 68.9, 63.5, 55.4, 52.4, 48.1, 43.9, 42.6, 35.5, 34.0, 30.0, 26.2, 22.7, 21.5, 19.5, 18.6, -5.1, -5.1.



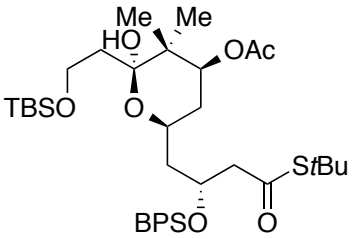
Preparation of (5*R*,7*S*,9*S*)-5-(2-(*tert*-butylthio)-2-oxoethyl)-7-((4-methoxybenzyl)oxy)-2,2,10,10,15,15,16,16-octamethyl-11-methylene-3,3-diphenyl-4,14-dioxa-3,15-disilaheptadecan-9-yl acetate 1.21.[111a] To a stirring solution of alcohol **1.48** (2.00 g, 2.39 mmol, 1.00 equiv), triethylamine (1 mL, 7.18 mmol, 3.00 equiv), DMAP (29.2 mg, 0.239 mmol, 0.10 equiv) in CH_2Cl_2 (24 mL, 0.10 M) in a 50 mL round-bottom flask under an atmosphere of N_2, at rt, was added acetic anhydride (452 μL, 4.78

mmol, 2.00 equiv) dropwise via syringe. TLC analysis after 18 h indicated complete consumption of alcohol starting material. The reaction mixture was diluted with 30% EtOAc/hexanes (100 mL), washed twice with a saturated aqueous $NaHCO_3$ solution (50 mL), once with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude product as pale yellow oil. Purification was accomplished by flash column chromatography on a (2.5 x 25) cm column, eluting with 5% EtOAc/ hexanes (1000 mL), collecting 10 mL fractions. The fractions containing product (31-69) were combined and concentrated under reduced pressure to give acetylated product **1.21** (1.90 g, 95%) as colorless oil: R_f 0.59 (30% EtOAc/hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.73 – 7.68 (m, 4H), 7.45 – 7.36 (m, 6H), 7.22 – 7.20 (m, 2H), 6.85 – 6.83 (m, 2H), 5.28 (d, *J* = 9.2 Hz, 1H), 4.90 (s, 1H), 4.83 (s, 1H), 4.28 – 4.23 (m, 1H), 4.21 (ABq, *J* = 10.3 Hz, Δν = 29.0 Hz, 2H), 3.80 (s, 3H), 3.67 (app t, *J* = 7.3 Hz, 2H), 3.25 – 3.19 (m, 1H), 2.65 (dd, *J* =14.6, 6.2 Hz, 1H), 2.55 (dd, *J* = 14.6, 6.2 Hz, 1H), 2.27 (ddd, *J* = 15.0, 7.3, 7.3 Hz, 1H), 2.22 (ddd, *J* = 15.0, 7.3, 7.3 Hz, 1H), 1.99 (s, 3H), 1.97 – 1.90 (m, 1H), 1.52 – 1.46 (m, 1H), 1.45 (s, 9H), 1.32 – 1.17 (m, 2H), 1.05 (s, 9H), 0.96 (s, 3H), 0.94 (s, 9H), 0.90 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.8, 170.9, 159.2, 150.9, 136.2, 136.1, 133.9, 131.1, 130.0, 129.9, 129.7, 127.9, 127.8, 113.8, 111.2, 74.6, 73.5, 71.3, 68.7, 63.4, 55.4, 52.4, 48.2, 43.5, 42.9, 35.8, 34.7, 30.0, 27.2, 26.2, 24.1, 21.7, 21.3, 19.6, 18.5, -5.0.



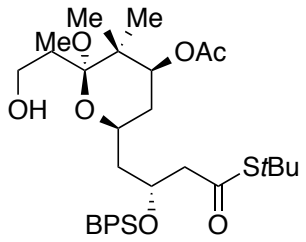
Preparation of (5*R*,7*S*,9*S*)-5-(2-(*tert*-butylthio)-2-oxoethyl)-7-hydroxy-

2,2,10,10,15,15,16,16-octamethyl-11-methylene-3,3-diphenyl-4,14-dioxa-3,15-disilaheptadecan-9-yl acetate 1.49.[111a] To a stirring mixture of PMB ether **1.21** (1.59 g, 1.81 mmol, 1.00 equiv) in CH_2Cl_2 (12 mL, 0.15 M), and pH 7.0 phosphate buffer (6.0 mL) in a 50 mL round-bottom flask under an atmosphere of N_2, at rt, was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (823 mg, 3.62 mmol, 2.00 equiv) in one portion. TLC analysis after 2 h indicated complete consumption of alcohol starting material. The reaction mixture was transferred to a 100 mL Erlenmeyer flask, containing 30% EtOAc/hexanes (50 mL), and filtered over a plug of Celite®, Florisil®, and Na_2SO_4. The plug was flushed with additional 30% EtOAc/hexanes (3 × 30 mL) portions. The filtrate was concentrated under reduced pressure to give the crude product as colorless oil. Purification was accomplished by flash column chromatography on a silica gel column (2.5 x 25) cm, eluting with 5% EtOAc/hexanes, collecting 10 mL fractions. The fractions containing product (64 – 77) were combined and concentrated under reduced pressure to give alcohol **1.49** (1.30 g, 95%) as colorless oil: R_f 0.15 (10% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.72 – 7.68 (m, 4H), 7.46 – 7.38 (m, 6H), 5.08 (dd, *J* = 11.4, 1.5 Hz, 1H), 4.94 (s, 1H), 4.87 (s, 1H), 4.43 – 4.38 (m, 1H), 3.72 (app dddd, *J* = 17.9, 9.9, 7.0, 7.0 Hz, 2H), 3.49 (app t, *J* = 8.8 Hz, 2H), 2.68 – 2.60 (m, 3H), 2.26 (app t, *J* = 7.3 Hz, 2H), 1.93 (s, 3H), 1.60 (ddd, *J* = 14.3, 9.2, 4.0 Hz, 1H), 1.55 (ddd, *J* = 14.3, 8.1, 2.6 Hz, 1H), 1.45 – 1.23 (m, 2H), 1.41 (s, 9H), 1.04 (s, 9H), 1.02 (s, 3H), 1.02 (s, 3H), 0.91 (s, 9H), 0.07 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 197.7, 172.2, 150.6, 136.1, 136.0, 133.8, 133.8, 130.0, 129.9, 127.9, 111.3, 75.2, 68.4, 63.8, 63.3, 52.5, 48.1, 44.4, 42.9, 38.1, 34.7, 29.9, 27.2, 26.2, 24.1, 22.0, 21.0, 19.6, 18.5, -5.1.



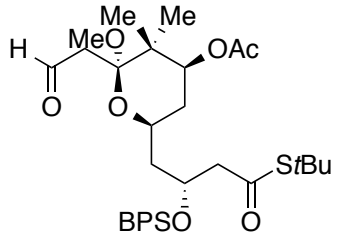
Preparation of (2*S*,4*S*,6*S*)-2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-hydroxy-3,3-dimethyltetrahydro-2*H*-pyran-4-yl acetate 1.50.[111a] To a stirring solution of alkene **1.49** (1.27 g, 1.67 mmol, 1.00 equiv) in CH_2Cl_2 (33.5 mL, 0.05 M) in a 100 mL round-bottom flask, at -78 °C, was bubbled a steady stream of ozone. After 10 min, during which time the solution color changed from colorless to dark blue, ozone bubbling was ceased, completion of the reaction was judged by TLC analysis and then replaced with a steady stream of N_2 for 5 min to purge the solution. Methyl sulfide (33.5 mL) was added to the reaction mixture and the resulting solution was stirred for 14 h. The stir bar was removed and the reaction mixture was concentrated under reduced pressure to give the crude product as yellow oil. Purification was accomplished by flash column chromatography on a silica gel column (2.5 x 25) cm, eluting with 5% EtOAc/hexanes (500 mL), collecting 10 mL fractions. The fractions containing product (11 – 33) were combined and concentrated under reduced pressure to give cyclic hemiketal **1.50** (1.13 g, 90%) as colorless oil: R_f 0.20 (10% EtOAc/hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.74 – 7.69 (m, 4H), 7.45 – 7.36 (m, 6H), 5.23 (s, 1H), 5.07 (dd, *J* = 11.7, 4.8 Hz, 1H) 4.31 (dddd, *J* = 8.4, 5.9, 5.9, 5.9 Hz, 1H), 4.17 (ddd, *J* = 12.1, 10.3, 1.8 Hz, 1H), 3.81 (dddd, *J* = 10.6, 7.7, 5.1, 3.3 Hz, 1H), 3.73 (ddd, *J* = 10.3, 4.0, 2.9 Hz, 1H), 2.70 (app d, *J* = 6.2 Hz, 2H), 2.01 (s, 3H), 1.92 – 1.86 (m, 1H), 1.73 (td, *J* = 14.3, 8.1 Hz, 1H), 1.58 – 1.41 (m, 3H), 1.45 (s, 9H), 1.36 (ddd, *J* = 12.1, 4.8, 2.9 Hz, 1H), 1.04 (s, 9H), 0.92 (s, 3H),

0.90 (s, 9H), 0.83 (s, 3H), 0.10 (s, 3H), 0.10 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 170.6, 136.1, 136.1, 129.9, 129.7, 127.8, 127.7, 102.3, 73.4, 69.3, 64.7, 60.3, 52.5, 48.1, 43.8, 41.3, 34.1, 33.5, 30.0, 27.2, 26.0, 21.4, 21.2, 19.6, 18.2, 16.8, -5.2, -5.4.



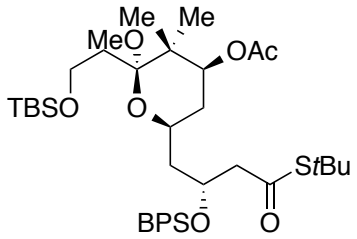
Preparation of (2*S*,4*S*,6*S*)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-(2-hydroxyethyl)-2-methoxy-3,3-dimethyltetrahydro-2*H*-pyran-4-yl acetate 1.51.[111a] To a stirring solution of hemiketal **1.50** (1.13 g, 1.48 mmol, 1.00 equiv) in MeOH (37.0 mL, 0.04 M) in a 100 mL round-bottom flask, at rt, was added (±)-camphor-10-sulfonic acid (86 mg, 0.37 mmol, 0.25 equiv) in one portion, under a steady stream of N_2. The reaction was allowed to proceed for 2 h, after which time TLC analysis indicated complete consumption of starting material. The reaction mixture was diluted with 50% EtOAc/hexanes (100 mL), quenched by the addition of a saturated aqueous $NaHCO_3$ solution (50 mL), and then the resulting phases were separated. The organic phase was washed twice with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a silica gel column (2.5 x 20) cm, eluting with 30% EtOAc/hexanes, collecting 10 mL fractions. The fractions containing product (22 - 34) were combined and concentrated under reduced pressure to give cyclic ketal **1.51** (1.13 g, quant.) as colorless oil: R_f 0.18 (30% EtOAc/hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.64 (m, 4H), 7.47 – 7.37 (m, 6H), 5.06 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.28 (quin, *J* = 5.9 Hz, 1H), 3.69 – 3.59 (m, 2H), 3.48 (dddd, *J* = 11.7, 7.7, 4.4, 3.3 Hz, 1H), 2.97 (s, 3H),

2.72 (dd, *J* = 14.6, 6.2 Hz, 1H), 2.62 (dd, *J* = 14.6, 6.2 Hz, 1H), 2.30 (t, *J* = 6.2 Hz, 1H), 2.05 – 1.96 (m, 1H), 2.03 (s, 3H), 1.84 – 1.73 (m, 2H), 1.53 – 1.41 (m, 2H), 1.44 (s, 9H), 1.13 – 1.10 (m, 1H), 1.04 (s, 9H), 0.91 (s, 3H), 0.83 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 170.7, 136.1, 136.0, 134.0, 133.8, 130.0, 130.0, 127.9, 104.9, 73.4, 68.8, 66.0, 59.8, 52.6, 48.7, 48.4, 43.9, 42.0, 34.7, 33.0, 29.9, 27.1, 21.4, 20.5, 19.5, 17.3.



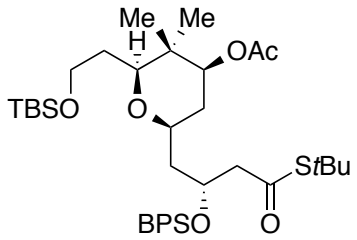
Preparation of (2*S*,4*S*,6*S*)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyl-2-(2-oxoethyl)tetrahydro-2*H*-pyran-4-yl acetate 1.20.[111a] To a stirring solution of alcohol **1.51** (99.7 mg, 0.151 mmol, 1.00 equiv) in CH_2Cl_2 (3.0 mL, 0.05 M) in a 15 mL round-bottom flask under an atmosphere of N_2, at -10 ^oC, was added N,N-diisopropylethylamine (136.5 mg, 1.06 mmol, 7.00 equiv). After 10 min at -10 ^oC, dimethyl sulfoxide (107.0 μL, 1.51 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was allowed to stir for additional 10 min. Sulfur trioxide pyridine complex (96.2 mg, 0.605 mmol, 4.00 equiv) was then added in one portion The reaction was allowed to proceed for 1 h at -10 ^oC, after which time TLC analysis indicated complete consumption of the starting material. The reaction was quenched by adding saturated aqueous solution of $NaHCO_3$ (10 mL) and the phases were separated. The aqueous phase was extracted with 20% EtOAc/ hexanes (3 × 30 mL). The combined organic phase was washed twice with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure Purification was by flash column chromatography on a silica gel column (10 × 1) cm eluting with 10%

EtOAc/ hexanes (300 mL), collecting 5 mL fractions. The fractions containing product (25 – 35) were concentrated under reduced pressure to give the aldehyde **1.20** (87.5 mg, 88%) as colorless oil: R_f 0.55 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 9.66 (t, *J* = 3.1 Hz, 1H), 7.73 – 7.66 (m, 4H), 7.50 – 7.37 (m, 6H), 5.02 (dd, *J* = 11.5, 4.6 Hz, 1H), 4.28 (quin, *J* = 6.6 Hz, 1H), 3.42 (dddd, *J* = 11.5, 7.6, 4.0, 4.0 Hz, 1H), 3.00 (s, 3H), 2.73 (dd, *J* = 14.6, 6.6 Hz, 1H), 2.64 (dd, *J* = 14.6, 5.5 Hz, 1H), 2.53 (app t, *J* = 3.1 Hz, 2H), 2.03 (s, 3H), 1.78 (quin, *J* = 7.1 Hz, 1H), 1.51 (td, *J* = 14.1, 4.4 Hz, 1H), 1.46 – 1.41 (m, 1H), 1.44 (s, 9H), 1.13 – 1.00 (m, 1H), 1.04 (s, 9H), 0.88 (s, 3H), 0.83 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.5, 197.9, 170.6, 136.1, 136.0, 134.1, 133.7, 130.1, 130.0, 127.9, 104.1, 72.8, 69.1, 66.4, 53.0, 48.8, 48.3, 45.8, 43.5, 42.0, 32.9, 30.0, 27.2, 21.4, 20.8, 19.5, 17.5.



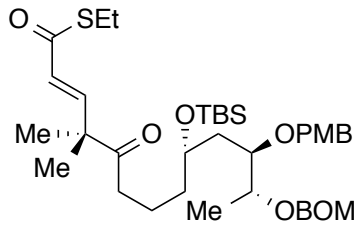
Preparation of (2*S*,4*S*,6*S*)-2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2*H*-pyran-4-yl acetate 1.52. To a stirring solution of the alcohol **1.51** (127.6 mg, 0.193 mmol, 1.00 equiv), imidazole (33.0 mg, 0.484 mmol, 2.50 equiv), DMAP (2.40 mg, 0.019 mmol, 0.10 equiv) in DMF (968 μL, 0.20 M) in a 5 mL round-bottom flask in the atmosphere of N_2, at rt, was added TBSCl (73 mg, 0.484 mmol, 2.50 equiv) in one portion. After 12 h, the reaction was judged complete by TLC analysis. The reaction was quenched by addition of saturated aqueous solution of $NaHCO_3$ (5 mL). The layers were separated and the organic layer was extracted with CH_2Cl_2 (3 × 50 mL),

washed once with brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a (1.5 × 15) cm silica gel column, eluting with 5% EtOAc/hexanes (250 mL), collecting 5 mL fractions. The fractions containing product (11-15) were combined and concentrated under reduced pressure to give the TBS ether **1.52** (173 mg, quant.) as colorless oil: R_f 0.76 (30% EtOAc/ hexanes); $[\alpha]^D_{20}$ = + 15.9 (*c* = 1.06, $CHCl_3$); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.65 (m, 4H), 7.46 – 7.36 (m, 6H), 4.96 (dd, *J* = 11.8, 4.9 Hz, 1H), 4.26 (m, 1H), 3.70 – 3.63 (m, 1H), 3.57 (dddd, *J* = 15.7, 10.3, 5.7, 5.7 Hz, 1H), 3.20 (m, 1H), 2.96 (s, 3H), 2.73 – 2.71 (m, 1H), 2.00 (s, 3H), 1.93 – 1.78 (m, 2H), 1.68 (ddd, J = 16.1, 8.0 Hz, 1H), 1.45 (s, 9H), 1.33 – 1.28 (m, 1H), 1.03 (s, 9H), 0.89 (s, 12H), 0.82 (s, 3H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.0, 170.7, 136.2, 136.0, 134.5, 133.8, 130.0, 129.8, 127.9, 127.8, 104.1, 73.8, 69.8, 66.0, 59.6, 53.3, 48.6, 48.2, 43.8, 41.7, 36.1, 33.0, 30.0, 27.1, 26.2, 21.4, 20.5, 19.6, 18.5, 17.4, -5.00, -5.01; 125 MHz DEPT ($CDCl_3$) δ CH_3: 48.2, 30.0, 27.1, 26.2, 21.4, 20.5, 17.4, -5.00, -5.01, CH_2: 59.6, 53.3, 43.8, 36.1, 33.0, CH: 136.2, 136.0, 130.0, 129.8, 127.9, 127.8, 73.8, 69.8, 66.0; IR (thin film) 3458, 3002, 2963, 1745, 1682, 1473, 1425, 1384, 1365, 1111, 650 cm^{-1}. HRMS (ESI/TOF) calcd for $C_{42}H_{68}O_7SSi_2Na$ *m*/*z* ($M + Na^+$) 795.4116. Found 795.4125.



Preparation of (2*S*,4*S*,6*S*)-2-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-3,3-dimethyltetrahydro-2*H*-pyran-4-yl acetate 1.53. To a stirring solution of ketal **1.52** (28.4 mg, 0.37 mmol,

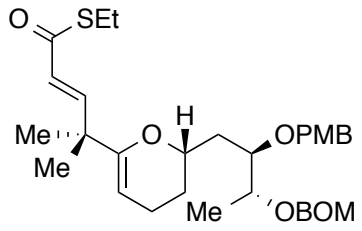
1.00 equiv), Et_3SiH (117 *m*L, 0.734 mmol, 20.0 equiv) in CH_2Cl_2 (734 μL, 0.05 M) in a 5 mL reaction vial in an atmosphere of N_2, at -78 ^oC, was added TMSOTf (124 μL, 0.11 mmol, 3.00 equiv) slowly down the inside of the vial. The reaction was quenched with the addition of Hünig's base (2 mL) at -78 ^oC. The cold bath was removed and the reaction mixture was stirred at rt for 15 min. The reaction mixture was then diluted with 30% EtOAc/hexanes (10 mL) and then washed twice with water (10 mL), once with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a (1 x 10) cm silica gel column, eluting with 5% EtOAc/ hexanes (100 mL), collecting 5 mL fractions. The fractions containing product (13-16) were combined, and concentrated under reduced pressure to give the C_9 deoxygenated A ring compound **1.53** (18 mg, 66%) as colorless oil: R_f = 0.48 (15% EtOAc/ hexanes); $[\alpha]^D_{20}$ = + 22.9 (*c* = 1.01, $CHCl_3$); 400 MHz ^1H NMR ($CDCl_3$) δ 7.73 – 7.65 (m, 4H), 7.45 – 7.36 (m, 6H), 4.45 (dd, *J* = 11.6, 4.8, 1H), 4.34 – 4.26 (m, 1H), 3.69 – 3.63 (m, 1H), 3.52 (m, 1H), 3.09 (m, 1H), 2.97 – 2.89 (m, 1H), 2.68 – 2.67 (m, 2H), 2.03 (s, 3H), 1.74 – 1.56 (m, 2H), 1.44 (s, 9H), 1.03 (s, 9H), 0.89 (s, 9H), 0.79 (s, 3H), 0.74 (s, 3H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 198.9, 170.7, 136.1, 136.0, 136.0, 134.6, 134.5, 133.9, 130.0, 129.9, 129.8, 127.8, 127.7, 80.2, 77.5, 72.8, 69.4, 60.8, 52.8, 48.1, 43.9, 37.4, 34.0, 32.7, 30.0, 27.2, 27.2, 27.1, 26.2, 22.4, 21.3, 19.6, 18.5, 13.7, 7.00, 4.61, -4.98; 125 MHz DEPT ($CDCl_3$) d CH_3: 30.0, 27.2, 27.1, 26.2, 22.4, 21.3, 13.7, 7.00, -4.98, CH_2: 60.8, 52.8, 43.9, 34.0, 32.7, 4.61, CH: 136.1, 136.0, 129.9, 129.8, 127.8, 127.7, 80.2, 77.5, 72.8, 69.4; IR (thin film) 3450, 3000, 2961, 2859, 1741, 1682, 1473, 1425, 1386, 1363, 1236, 1111, 698 cm^{-1}. HRMS (ESI/TOF) calcd for $C_{41}H_{66}O_6SSi_2Na$ ($M + Na^+$) 765.4011. Found 765.4019.



Preparation of *S*-ethyl (9*S*,11*R*,12*R*,*E*)-12-((benzyloxy)methoxy)-9-((*tert*-butyldimethylsilyl)oxy)-11-((4-methoxybenzyl)oxy)-4,4-dimethyl-5-oxotridec-2-enethioate 1.56.[102c] To a solution of alkene **1.29** (1.76 g, 2.87 mmol, 1.0 equiv) in a 20:1 EtOAc/MeOH mixture (57.4 mL, 0.05M) in a 100 mL round-bottom flask, at -78 °C, was added $NaHCO_3$ (2.40 g, 28.7 mmol, 10.0 equiv). A steady stream of O_3 was bubbled through the reaction mixture until a light blue color developed. The excess O_3 was removed by bubbling O_2 through the mixture for 15 min until the light blue color faded. A solution of PPh_3 (1.13 g, 4.30 mmol, 1.5 equiv) in CH_2Cl_2 (5 mL) was added to the reaction mixture, and the mixture was slowly warmed to rt with stirring. After 12 h, the mixture was filtered, and concentrated under reduced pressure. The resulting yellow oil was taken-up in 10% Et_2O/pentane (100 mL) in a 500 mL round-bottom flask, and placed in a -20 °C freezer for 6 h. The triphenylphosphine oxide precipitate was removed via filtration, and rinsed with ice cold 1% Et_2O/pentane (50 mL). The solvent was removed under reduced pressure to provide crude aldehyde **1.54** as a light yellow oil, which was taken into the next step without further purification; R_f = 0.51 (30% EtOAc/hexanes).

To a stirring solution of *S*-ethyl 2-(diethoxyphosphoryl)ethanethioate (**1.55**) (1.52 g, 6.31 mmol, 2.2 equiv) in THF (16 mL, 0.18M) in a 50 mL round-bottom flask at 0 °C, was added NaH (151.5 mg, 6.31 mmol, 2.2 equiv) slowly over 10 min. The reaction mixture was stirred at 0 °C for an addition of 30 min, and then a solution of the previously mentioned crude aldehyde **1.54** (assumed to be 2.87 mmol) in THF (5 mL)

was added slowly via cannula. The transfer was completed by rinsing with THF (2 × 2 mL). Stirring was continued at 0 °C for an addition 2 h, and the reaction mixture was then quenched by the addition of saturated aqueous NH_4Cl solution (20 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography, eluting with 20% EtOAc/hexanes. The fractions containing product were combined and concentrated under reduced pressure to provide thioester **1.56** (1.71 g, 85%) as clear colorless oil: R_f = 0.63 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR (CDCl3) δ 7.40-7.35 (m, 4H), 7.35-7.25 (m, 1H), 7.25-7.23 (m, 2H), 6.98 (d, *J* = 15.9 Hz, 1H), 6.88-6.83 (m, 2H), 6.15 (d, *J* = 15.9 Hz, 1H), 4.81 (d, *J* = 7.3 Hz, 1H), 4.79 (d, *J* = 7.3 Hz, 1H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 10.6 Hz, 1H), 4.44 (d, *J* = 10.6 Hz, 1H), 4.01 (qd, *J* = 6.4, 4.7 Hz, 1H), 3.89 (m, 1H), 3.80 (s, 3H), 3.63 (ddd, *J* = 9.4, 4.5, 2.3 Hz, 1H), 2.97 (q, *J* = 7.4 Hz, 2H), 2.41(m, 2H), 1.68 (ddd, *J* = 15.1, 8.5, 2.6 Hz, 1H), 1.61-1.53 (m, 3H), 1.46-1.40 (m, 2H), 1.30(t, *J* = 7.5 Hz, 3H), 1.27 (s, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 3H), 0.04 (s, 3H); 125 MHz ^{13}C NMR (CDCl3) δ 210.7, 190.1, 160.0, 147.4, 138.2, 131.2, 129.8, 129.3, 128.6, 128.0, 127.9, 127.7, 114.0, 93.4, 78.2, 73.0, 72.1, 69.6, 69.5, 55.5, 50.7, 38.6, 37.8, 37.4, 26.2, 23.7, 23.5, 19.2, 18.3, 15.3, 14.9, -3.6, -4.2.

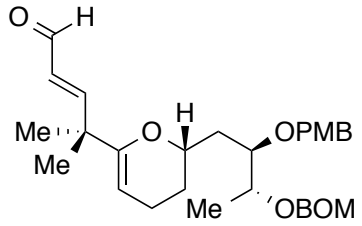


Preparation of *S*-ethyl (*E*)-4-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-

methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-4-methylpent-2-enethioate 1.57.[102c] To a stirring solution of thioester **1.56** (91.4 mg, 0.130 mmol, 1.0 equiv) in 20:1 CH_3CN/water (2.6 mL, 0.05 M), in a 50 mL high density polyethylene bottle at 0 °C, were added pyridine (434 μL), and aqueous HF soln. (48%, 200 μL). (Caution: laboratory coats, proper gloves should be worn) The solution was stirred at 0 °C for 30 min and was then warmed to rt. After 30 min of stirring at rt, additional aqueous HF solution (48%, 200 μL) was added every hour until TLC analysis indicated complete consumption of the starting material. The reaction mixture was quenched by carefully transferring the reaction mixture into a stirring mixture of saturated aqueous $NaHCO_3$ solution (50 mL) and EtOAc (50 mL) via plastic pipette. Solid $NaHCO_3$ was then added until effervescence was complete. The phases were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with water (3 × 20 mL), saturated aqueous $CuSO_4$ solution (2 × 20 mL), and brine (3 × 20 mL). The solution was dried over Na_2SO_4, filtered, and concentrated to provide the intermediate alcohol as clear light yellow oil. This material was carried into the next step without further purification.

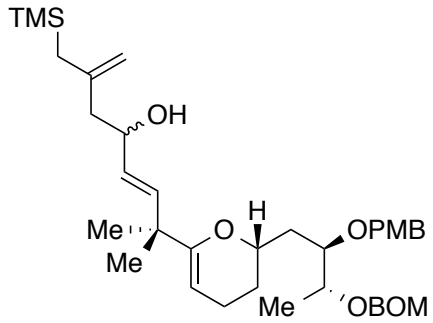
To a stirring solution of the previously described intermediate alcohol (assumed to be 0.130 mmol) in benzene (5.0 mL, 0.03 M), in a 25 mL round-bottom flask equipped with a condenser and Dean-Stark trap, was added CSA (1 mg, 0.002 mmol, 0.05 equiv). The solution was heated at reflux for 1 h, and was then allowed to cool to rt. The reaction mixture was quenched by the addition of pyridine (0.1 mL), and the mixture was then concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a (1 × 10) cm silica gel column, eluting with 5-8% EtOAc/hexanes,

collecting 9 mL fractions. The fractions containing product (15-60) were combined and concentrated under reduced pressure to yield dihydropyran **1.57** (67 mg, 90%) as clear colorless oil: R_f = 0.56 (30% EtOAc/ hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.34 (m, 4H), 7.33-7.28 (m, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 15.9 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 1H), 6.09 (d, *J* = 15.9 Hz, 1H), 4.85 (d, *J* = 6.9 Hz, 1H), 4.82 (d, *J* = 6.9 Hz, 1H), 4.68-4.59 (m, 4H), 4.49 (d, *J* = 11.1 Hz, 1H), 4.04-3.94 (m, 2H), 3.84-3.78 (m, 4H), 2.89 (q, *J* = 7.4 Hz, 2H), 2.14-2.04 (m, 1H), 2.03-1.96 (m, 1H), 1.84-1.73 (m, 2H), 1.65-1.58 (m, 1H), 1.55-1.46 (m, 1H), 1.24-1.19 (m, 12H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 190.6, 159.3, 157.1, 151.9, 138.2, 131.1, 129.7, 128.6, 128.0, 127.8, 125.7, 113.9, 94.6, 93.6, 77.7, 73.9, 73.6, 72.0, 69.6, 55.5, 41.4, 36.2, 28.2, 25.3, 25.2, 23.3, 20.5, 15.6, 14.9; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.5, 25.3, 25.2, 15.6, 14.9; CH_2 δ 93.6, 73.6, 69.6, 36.2, 28.2, 23.3, 20.5; CH δ 151.9, 129.7, 128.6, 128.0, 127.8, 125.7, 113.9, 94.6, 77.7, 73.9, 72.0.



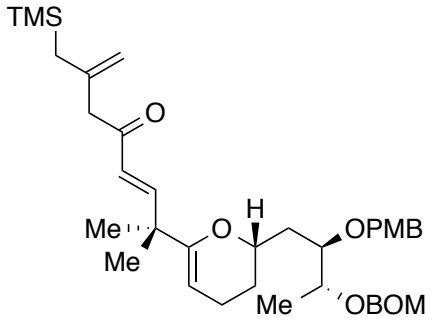
Preparation of (*E*)-4-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-4-methylpent-2-enal 1.27.[102c] To a stirring solution of thioester **1.57** (1.28 g, 2.25 mmol, 1 equiv) in CH_2Cl_2 (22.5 mL, 0.1 M) at - 78 ^{o}C was added a solution of DIBAL-H in toluene (4.5 mL of 1.5 M, 1.1 mmol, 3 equiv) dropwise over a period of 30 min. This mixture was stirred at -78 ^{o}C for 1.5 h, then EtOAc (2 mL) was added dropwise over 10 min. The solution was stirred for 15 min, and then quenched by the addition of saturated aqueous Rochelle salt (10 mL)

dropwise. The cold bath was removed, and the mixture was stirred vigorously for 12 h at ambient temperature. The layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via flash column chromatography, eluting with 10% EtOAc/hexanes (300 mL and 15% EtOAc/hexanes (250 mL) collecting 9 mL fractions. The fractions containing product (35-65) were collected and concentrated under reduced pressure to provide α,β-unsaturated aldehyde **1.27** (893 mg, 78%) as colorless oil: R_f = 0.54 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.47 (d, *J* = 7.8 Hz, 1H), 7.37 – 7.23 (m, 8H), 6.87 – 6.83 (m, 4H), 6.10 (dd, *J* = 16.1, 7.81 Hz, 1H), 4.81 (ABq, = 10.7 Hz, 2H), 4.62 – 4.60 (m, 4H), 4.46 (d, *J* = 10.7 Hz, 1H), 4.05 – 3.94 (m, 2H), 3.80 – 3.75 (m, 1H), 3.79 (s, 3H), 2.13 - 2.06 (m, 1H), 2.03 – 1.97 (m, 1H), 1.83 – 1.76 (m, 2H), 1.64 – 1.58 (m, 1H), 1.55 – 1.47 (m, 1H), 1.25 (d, *J* = 3.4 Hz, 6H), 1.20 (d, *J* = 6.3 Hz, 3H); 125 MHz ^{13}C NMR (CDCl3) δ 194.5, 165.5, 159.3, 156.6, 138.0, 130.8, 129.7, 129.4, 128.5, 127.9, 127.7 113.9, 94.7, 93.5, 77.5, 73.5, 73.2, 72.0, 69.5, 55.3, 42.0, 35.1, 28.0, 25.2, 25.0, 20.4, 15.4.



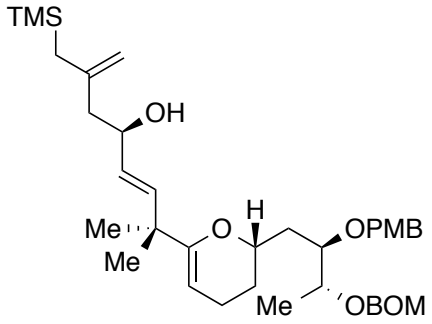
Preparation of (*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-7-methyl-2-

((trimethylsilyl)methyl)octa-1,5-dien-4-ol 1.58.[124] To a solution of aldehyde **1.27** (55.2 mg, 0.108 mmol, 1 equiv) in toluene (362 μL, 0.30 M) in a 5 mL round-bottom flask was added a trimethyl(2-((tributylstannyl)methyl)allyl)silane **1.28** via syringe. The mixture was heated to reflux at 120 ^{o}C for 24 h in which TLC showed the completion of reaction. The reaction mixture was allowed to cool to rt and the solvent was removed under reduced pressure. Purification was accomplished using flash column chromatography with a 1 x 10 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product (11-26) were combined and concentrated under reduced pressure to provide alcohol **1.58** (62 mg, 90%) as 1:1 mixture of diastereomers as colorless oil. R_f = 0.57 (30% EtOAc/hexanes); 500 MHz 1H NMR (C_6D_6) δ 7.38-7.08 (m, 7H), 6.82-6.80 (m, 2H), 6.04 (dd, *J* = 15.6, 6.3 Hz, 1H), 5.67 (dd, *J* = 15.6, 5.8 Hz, 1H), 4.83-4.70 (m, 4H), 4.67-4.6 (m, 4H), 4.29-4.4.05 (m, 4H), 3.30 (s, 3H), 2.59-2.50 (m, 2H), 2.29-2.15 (m, 2H), 2.02- 1.83 (m, 5H), 1.76-1.71 (m, 1H), 1.64-1.38 (m, 5H), 1.33 (dd, *J* = 8.7, 2.4 Hz, 6H), 1.23 (dd, *J* = 6.3 Hz, 4H), 0.00 (s, 9H).



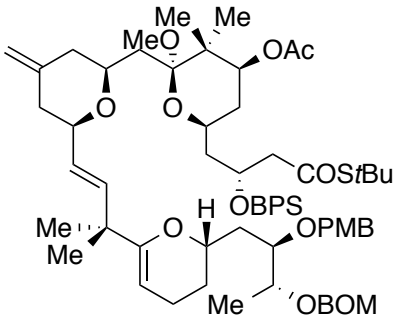
Preparation of (*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-7-methyl-2-((trimethylsilyl)methyl)octa-1,5-dien-4-one 1.59.[124] To a stirring solution of alcohol **1.58** (35.6 mg, 0.056 mmol, 1.0 equiv) in CH_2Cl_2 (558 μL, 0.1 M) in a 10 mL round-bottom flask at -15 °C was added freshly distilled N,N-diisopropylethylamine (68 μL,

0.391 mmol, 7.0 equiv) dropwise via syringe. After 10 min at -15 °C, dimethyl sulfoxide (39.5 μL, 0.558 mmol, 10.0 equiv) was added to the reaction mixture via syringe and the solution was stirred for an additional 10 min. Sulfur trioxide pyridine complex (35.5 mg, 0.223 mmol, 4.0 equiv) was then added in one portion. The reaction mixture was allowed to proceed for 1 h at -15 °C, after which time TLC analysis indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (10 mL), quenched by addition of saturated aqueous $NaHCO_3$ solution (2 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 × 10 cm column, eluting with 10% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product (20-55) were combined and concentrated under reduced pressure to give the ketone **1.59** (31 mg, 88% yield) as colorless oil: R_f = 0.53 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR (C_6D_6) δ 7.39-7.09 (m, 7H), 6.83-6.81 (m, 2H), 6.34 (d, *J* = 15.6 Hz, 1H), 4.81-4.75 (m, 4H), 4.69 (d, *J* = 10.7 Hz, 2H), 4.63 (d, *J* = 2.9 Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 2H), 4.53 (t, *J* = 3.9 Hz, 1H), 4.14-4.00 (m, 3H), 3.30 (s, 3H), 3.11 (s, 2H), 1.98-1.89 (m, 1H), 1.86 (ddd, *J* = 9.2, 6.8, 2.4 Hz, 1H), 1.81-1.75 (m, 1H), 1.61-1.56 (m, 3H), 1.51-1.47 (m, 1H), 1.41-1.32 (m, 1H), 1.23 (s, 3H), 1.22 (d, J = 4.3 Hz, 3H), 1.19 (s, 3H), -0.00 (s, 9H); 125 MHz 13C NMR (C_6D_6) δ 197.0, 160.4, 157.9, 154.1, 142.3, 139.2, 132.0, 129.8, 128.9, 128.3, 128.0, 126.4, 114.4, 112.4, 94.9, 93.8, 77.9, 73.6, 73.5, 72.7, 69.7, 55.1, 41.8, 36.3, 28.7, 27.6, 25.6, 25.6, 21.0, 15.4, -0.9.



Preparation of (*R*,*E*)-7-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-7-methyl-2-((trimethylsilyl)methyl)octa-1,5-dien-4-ol 1.19.[124] A stirring solution of CBS•BH_3 (8.97 mg, 0.031 mmol, 1.1 equiv) in CH_2Cl_2 (280 μL, 0.1 M) was cooled to -40 ^oC. To this solution was added a solution of ketone **1.59** (17.8 mg, 0.028 mmol, 1 equiv) in 60 μL of toluene dropwise via syringe pump down the side of the flask over 15 min. The mixture was stirred at -40 ^oC for 1 h, after which it was quenched by slow addition of MeOH. The reaction was allowed to come to rt over 10-15 min and the solvent was evaporated under reduced pressure. Purification was accomplished using flash column chromatography with a 0.5 x 10 cm silica gel column, eluting with 10% EtOAc/hexanes, collecting 5 ml fractions. The fractions containing product (45-60) were combined and concentrated under reduced pressure to provide the alcohol **1.19** (15 mg, 85%) as a clear colorless oil: R_f = 0.31 (20% EtOAc/hexanes); 500 MHz ^1H NMR (C_6D_6) δ 7.39-7.10 (m, 7H), 6.82-6.80 (m, 2H), 6.05 (d, *J* = 16.4 Hz, 1H), 5.67 (dd, *J* = 15.6, 6.3 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 2H), 4.77 (d, *J* = 6.8 Hz, 1H), 4.74 (s, 1H), 4.73 (d, *J* = 11.2 Hz, 1H), 4.68-4.67 (m, 3H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.29 (dd, *J* = 12.6, 6.5 Hz, 1H), 4.22-4.18 (m, 1H), 4.12 (td, *J* = 4.9, 1.9 Hz, 1H), 4.10-4.05 (m, 1H), 3.29 (s, 3H), 2.29-2.21 (m, 2H), 1.99 (ddd, *J* = 13.6, 10.2, 1.4 Hz, 1H), 1.94 (ddd, J = 9.7, 6.8, 2.4 Hz, 1H), 1.89-1.83 (m, 1H), 1.77 (s, 1H), 1.60 (ddd, *J* = 13.1, 10.7, 2.4 Hz, 1H), 1.56-1.53 (m, 1H), 1.50 (d, *J* = 2.9 Hz, 3H),

1.47-1.37 (m, 2H), 1.34 (s, 3H), 1.33 (s, 3H), 1.23 (d, *J* = 6.3 Hz, 3H), -0.00 (s, 9H).



Preparation of (2*S*,4*S*,6*S*)-2-(((2*S*,6*R*)-6-((*E*)-3-((*S*)-2-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-3,4-dihydro-2*H*-pyran-6-yl)-3-methylbut-1-en-1-yl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-6-((*R*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-(*tert*-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyltetrahydro-2*H*-pyran-4-yl acetate 1.62.[124] To a stirring solution of aldehyde **1.20** (85.7 mg, 0.130 mmol, 1.1 equiv) and hydroxyallylsilane **1.19** (75.5 mg, 0.118 mmol, 1.0 equiv) in Et_2O (2.4 mL, 0.05 M) in a flame dried 15 mL round-bottom flask at -78 ^oC was added a solution of TMSOTf in Et_2O (142 μL, 1.0 M, 0.142 mmol, 1.2 equiv). After 1.5 h at - 78 ^oC, the reaction was quenched by addition of diisopropylethylamine (0.2 mL), followed by addition of saturated aqueous $NaHCO_3$ solution (2 mL). The mixture was warmed to rt, the phases were separated, and the aqueous phase was extracted with Et_2O (2 × 15 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 × 10 cm silica gel column, eluting with hexanes/EtOAc (9:1), collecting 5 mL fractions. The fractions containing product (17-62) were combined and concentrated under reduced pressure to provide the pyran **1.62** (78.5 mg, 55%) as white foam: R_f = 0.56 (30% EtOAc/hexanes); 500 MHz 1H NMR (C_6D_6) δ 7.83-7.80 (m, 4H), 7.38-7.09 (m, 13H), 6.82-6.79 (m, 2H), 6.08 (dd, *J* = 15.6, 0.9 Hz, 1H), 5.71 (dd, *J* =

16.1, 5.3 Hz, 1H), 5.35 (dd, *J* = 11.7 Hz, 4.8, 1H), 4.88 (s, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.77 (d, *J* = 4.3 Hz, 1H), 4.71-4.66 (m, 3H), 4.64-4.63 (m, 4H), 4.57-4.54 (m, 1H), 4.20-4.16 (m, 1H), 4.08- 4.06 (m, 2H), 3.88-3.85 (m, 1H), 3.65-3.63 (m, 1H), 3.30 (s, 3H), 2.93 (s, 3H), 2.91-2.89 (m, 2H), 2.33-2.24 (m, 2H), 2.19-2.15 (m, 1H), 2.11 (t, *J* = 12.4 Hz, 1H), 2.01-1.94 (m, 2H), 1.91-1.76 (m, 4H), 1.72 (d, J = 3.9 Hz, 1H), 1.69-1.66 (m, 1H), 1.64 (s, 3H), 1.60- 1.52 (m, 4H), 1.43 (s, 9H), 1.34 (s, 3H), 1.33 (s, 3H), 1.23 (d, J = 6.3Hz, 3H), 1.19 (s, 9H), 1.07(s, 3H), 1.03 (s, 3H).

References

1. *Marine Microbiology: Bioactive Compounds and Biotechnological Applications*. Wiley: 2013.

2. Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B., *Nature* **1970**, *227*, 962-963.

3. (a) Haygood, M. G.; Davidson, S. K., *Appl. Environ. Microbiol.* **1997**, *63*, 4612-4616; (b) Davidson, S. K.; Haygood, M. G., *Biol. Bull.* **1999**, *196*, 273-280; (c) Davidson, S. K.; Allen, S. W.; Lim, G. E.; Anderson, C. M.; Haygood, M. G., *Appl. Environ. Microbiol.* **2001**, *67*, 4531-4537.

4. (a) Pettit, G. R.; Herald, C. L.; Kamano, Y., *J. Org. Chem.* **1983**, *48*, 5354-5356; (b) Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R., *J. Nat. Prod.* **1983**, *46*, 528-531; (c) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M., *J. Am. Chem. Soc.* **1984**, *106*, 6768-6771; (d) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J., *Tetrahedron* **1985**, *41*, 985-994; (e) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M., *Can. J. Chem.* **1985**, *63*, 1204-1208; (f) Pettit, G. R.; Kamano, Y.; Herald, C. L., *J. Nat. Prod.* **1986**, *49*, 661-664; (g) Pettit, G. R.; Kamano, Y.; Herald, C. L., *J. Org. Chem.* **1987**, *52*, 2848-2854; (h) Pettit, G. R.; Leet, J. E.; Herald, C. L.; Kamano, Y.; Boettner, F. E.; Baczynskyj, L.; Nieman, R. A., *J. Org. Chem.* **1987**, *52*, 2854-2860; (i) Pettit, G. R.; Gao, F.; Sengupta, D.; Coll, J. C.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Van Camp, J. R.; Rudloe, J. J.; Nieman, R. A., *Tetrahedron* **1991**, *47*, 3601-3610; (j) Pettit, G. R.; Gao, F.; Blumberg, P. M.; Herald, C. L.; Coll, J. C.; Kamano, Y.; Lewin, N. E.; Schmidt, J. M.; Chapuis, J.-C., *J. Nat. Prod.* **1996**, *59*, 286-289; (k) Lopanik, N.; Gustafson, K. R.; Lindquist, N., *J. Nat. Prod.* **2004**, *67*, 1412-1414; (l) Lin, H.; Yao, X.; Yi, Y.; Li, X.; Wu, H., *Zhongguo Haiyang Yaowu* **1998**, *17*, 1; (m) Lin, H.; Liu, G.; Yi, Y.; Yao, X.; Wu, H., *Dier Junyi Daxue Xuebao* **2004**, *25*, 473.

5. Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J., *J. Am. Chem. Soc.* **1982**, *104*, 6846-6848.

6. Pettit, G. R.; Herald, C. L.; Gao, F.; Sengupta, D.; Herald, C. L., *J. Org. Chem.* **1991**, *56*, 1337-1340.

7. Lopanik, N.; Lindquist, N.; Targett, N., *Oecologia* **2004**, *139*, 131-139.

8. Pettit, G. R.; Gao, F.; Herald, D. L.; Blumberg, P. M.; Lewin, N. E.; Nieman, R. A., *J. Am. Chem. Soc.* **1991**, *113*, 6693-6695.

9. Schmidt, J. M.; Pettit, G. R., *Experientia* **1978**, *34*, 659-660.

10. Pettit, G. R., The Bryostatins. In *Progress in the Chemistry of Organic Natural Products*, Herz, W.; Kirby, G. W.; Steglich, W.; Tamm, C., Eds. Springer Vienna: 1991; pp 153-195.

11. Jones, R. J.; Sharkis, S. J.; Miller, C. B.; Rowinsky, E. K.; Burke, P. J.; May, W. S., *Blood* **1990**, *75*, 1319-1323.

12. Kennedy, M. J.; Prestigiacomo, L. J.; Tyler, G.; May, W. S.; Davidson, N. E., *Cancer Res.* **1992**, *52*, 1278-1283.

13. Dale, I. L.; Gescher, A., *Int. J. Cancer* **1989**, *43*, 158-163.

14. Hornung, R. L.; Pearson, J. W.; Beckwith, M.; Longo, D. L., *Cancer Res.* **1992**, *52*, 101-107.

15. Spitaler, M.; Utz, I.; Hilbe, W.; Hofmann, J.; Grunicke, H. H., *Biochem. Pharmacol.* **1998**, *56*, 861-869.

16. (a) May, W. S.; Sharkis, S. J.; Esa, A. H.; Gebbia, V.; Kraft, A. S.; Pettit, G. R.; Sensenbrenner, L. L., *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8483-8487; (b) Heyworth, C. M.; Dexter, T. M.; Nicholls, S. E.; Whetton, A. D., *Blood* **1993**, *81*, 894-900.

17. Li, F.; Grant, S.; Pettit, G. R.; McCrady, C. W., *Blood* **1992**, *80*, 2495-2502.

18. (a) Sharkis, S. J.; Jones, R. J.; Bellis, M. L.; Demetri, G. D.; Griffin, J. D.; Civin, C.; May, W. S., *Blood* **1990**, *76*, 716-720; (b) Leonard, J. P.; May, W. S.; Ihle, J. N.; Pettit, G. R.; Sharkis, S. J., *Blood* **1988**, *72*, 1492-1496.

19. Drager, A. M.; Van der Hem, K. G.; Zevenbergen, A.; Odding, J. H.; Huijgens, P. C.; Schuurhuis, G. J., *Leukemia* **1999**, *13*, 62-69.

20. Trenn, G.; Pettit, G. R.; Takayama, H.; Hu-Li, J.; Sitkovsky, M. V., *J. Immunol.* **1988**, *140*, 433-439.

21. (a) Tuttle, T. M.; Inge, T. H.; Wirt, C. P.; Frank, J. L.; McCrady, C. M.; Bear, H. D., *J. Immunother.* **1992**, *12*, 75-81; (b) Tuttle, T. M.; Bethke, K. P.; Inge, T. H.; McCrady, C. W.; Pettit, G. R.; Bear, H. D., *J. Surg. Res.* **1992**, *52*, 543-548.

22. Carr, M. E., Jr.; Carr, S. L.; Grant, S., *Anticancer Drugs* **1995**, *6*, 384-391.

23. (a) Esa, A. H.; Warren, J. T.; Hess, A. D.; May, W. S., *Res. Immunol.* **1995**, *146*, 351-361; (b) Bosco, M. C.; Rottschafer, S.; Taylor, L. S.; Ortaldo, J. R.; Longo, D. L.; Espinoza-Delgado, I., *Blood* **1997**, *89*, 3402-3411.

24. Mehla, R.; Bivalkar-Mehla, S.; Zhang, R.; Handy, I.; Albrecht, H.; Giri, S.; Nagarkatti, P.; Nagarkatti, M.; Chauhan, A., *PLoS ONE* **2010**, *5*, e11160.

25. (a) Stone, R. M.; Sariban, E.; Pettit, G. R.; Kufe, D. W., *Blood* **1988**, *72*, 208-213; (b) Steube, K. G.; Drexler, H. G., *Leuk. Lymphoma* **1993**, *9*, 141-148.

26. (a) Hu, Z. B.; Ma, W.; Uphoff, C. C.; Lanotte, M.; Drexler, H. G., *Leukemia* **1993**, *7*, 1817-1823; (b) Vrana, J. A.; Rao, A. S.; Wang, Z.; Jarvis, W. D.; Grant, S., *Int. J. Oncol.* **1998**, *12*, 927-934.

27. Elgie, A. W.; Sargent, J. M.; Alton, P.; Peters, G. J.; Noordhuis, P.; Williamson, C. J.; Taylor, C. G., *Leukemia Res.* **1998**, *22*, 373-378.

28. Drexler, H. G.; Quentmeier, H.; Dirks, W. G.; Uphoff, C. C.; Macleod, R. A. F., *Leukemia* **2002**, *16*, 1868-1870.

29. Mohammad, R. M.; Beck, F. W.; Katato, K.; Hamdy, N.; Wall, N.; Al-Katib, A., *Biol. Chem.* **1998**, *379*, 1253-1261.

30. (a) Basu, A.; Evans, R. W., *Int. J. Cancer* **1994**, *58*, 587-591; (b) Wang, Z.; Su, Z., -Z.; Fisher, P. B.; Wang, S.; VanTuyle, G.; Grant, S., *Exp. Cell Res.* **1998**, *244*, 105-116; (c) Al-Katib, A. M.; Smith, M. R.; Kamanda, W. S.; Pettit, G. R.; Hamdan, M.; Mohamed, A. N.; Chelladurai, B.; Mohammad, R. M., *Clin. Cancer Res.* **1998**, *4*, 1305-1314; (d) Koutcher, J. A.; Motwani, M.; Zakian, K. L.; Li, X.-K.; Matei, C.; Dyke, J. P.; Ballon, D.; Yoo, H.-H.; Schwartz, G. K., *Clin. Cancer Res.* **2000**, *6*, 1498; (e) Mohammad, R. M.; Wall, N. R.; Dutcher, J. A.; Katib, A. M., *Clin. Cancer Res.* **2000**, *6*, 4950-4956; (f) Basu, A.; Lazo, J. S., *Cancer Res.* **1992**, *52*, 3119-3124.

31. Mohammad, R. M.; Al-Katib, A.; Pettit, G. R.; Sensenbrenner, L. L., *Cancer Res.* **1994**, *54*, 165-168.

32. Zonder, J. A.; Philip, P. A., *Exp. Opin. Invest. Drugs* **1999**, *8*, 2189-2199.

33. (a) Maki, A.; Diwakaran, H.; Redman, B.; Al-Asfar, S.; Pettit, G. R.; Mohammad, R. M.; Al-Katib, A., *Anticancer Drugs* **1995**, *6*, 392-397; (b) Mohammad, R. M.; Pettit, G. R.; Almatchy, V. P.; Nathan, W.; Varterasian, M. L.; Al-Katib, A., *Anticancer Drugs* **1998**, *9*, 149-156; (c) Mohammad, R. M.; Varterasian, M. L.; Almatchy, V. P.; Hannoudi, G. N.; Pettit, G. R.; Al-Katib, A., *Clin. Cancer Res.* **1998**, *4*, 1337-1343; (d) McGown, A. T.; Jayson, G.; Pettit, G. R.; Haran, M. S.; Ward, T. H.; Crowther, D., *Br. J. Cancer* **1998**, *77*, 216-220.

34. (a) Grant, S.; Pettit, G. R.; McCrady, C. M., *Exp. Hematol.* **1992**, *20*, 34-42; (b) Watson, N. C.; Jarvis, W. D.; Orr, M. S.; Grant, S.; Gewirtz, D. A., *Int. J. Radiat. Biol.* **1996**, *69*, 183-192.

35. (a) Sun, M.-K.; Alkon, D. L., *Eur. J. Pharmacol.* **2005**, *512*, 43-51; (b) Kuzirian, A. M.; Epstein, H. T.; Gagliardi, C. J.; Nelson, T. J.; Sakakibara, M.; Taylor, C. G.; Scioletti, A. B.; Alkon, D. L., *Biological Bulletin* **2006**, *210*, 201-214; (c) Sun, M.-K.; Alkon, D. L., *Eur. J. Pharmacol.* **2008**, *584*, 328-337.

36. Etcheberrigaray, R.; Tan, M.; Dewachter, I.; Kuiperi, C.; Van der Auwera, I.; Wera, S.; Qiao, L.; Baank, B.; Nelson, T., J.; Kozikowski, A. P.; Van Leuven, F.; Alkon, D. L., *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11141-11146.

37. Sun, M.-K.; Hongpaisan, J.; Nelson, T. J.; Alkon, D. L., *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13620-13625.

38. Philip, P. A.; Rea, D.; Thavasu, P.; Carmichael, J.; Stuart, N. S. A.; Rockett, H.; Talbot, D. C.; Ganesan, T.; Pettit, G. R.; Balkwill, F.; Harris, A. L., *J. Natl. Cancer Inst.* **1993**, *85*, 1812-1818.

39. (a) Kaubisch, A.; Kelsen, D. R.; Saltz, L.; Kemeny, N.; O'Reilly, E.; Ilson, D.; Endres, S.; Barazzuol, J.; Schwartz, G. K., *Proc. Am. Soc. Clin. Oncol.* **1999**, *18*, 639; (b) Rosenthal, M. A.; Oratz, R.; Liebes, L.; Cahr, M. H.; Muggia, F. M., *Proc. Am. Soc. Clin. Oncol.* **1999**, *18*, 873.

40. Newman, D. J., The Bryostatins. In *Anticancer agents from natural products*, 2011; pp 199-218.

41. For the latest information on clinical trials with bryostatin 1, please see. http://clinicaltrials.gov.

42. Berkow, R. L.; Kraft, A. S., *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116.

43. Inoue, M.; Kishimoto, A.; Takai, Y.; Nishizuka, Y., *J. Biol. Chem.* **1977**, *252*, 7610-7616.

44. Murray, N. R.; Thompson, L. J.; Fields, A. P., The role of protein kinase C in cellular proliferation and cell cycle control. In *Molecular Biology Intelliegence Unit*, Parker, P. J.; Dekker, L. V., Eds. R. G. Landes Co., Austin, TX: 1997; pp 97-120.

45. Lonne, G. K.; Larsson, C., In *Protein Kinase C in Cancer SIgnaling and Therapy*, Kazanietz, M. G., Ed. Springer Science+Business Media: 2010; pp 107-115.

46. Griner, E. M.; Kazanietz, M. G., *Nat. Rev. Cancer* **2007**, *7*, 281-294.

47. (a) Newton, A. C., *Biochem. J* **2003**, *370*, 361-371; (b) Newton, A. C., In *Protein Kinase C in Cancer SIgnaling and Therapy*, Kazanietz, M. G., Ed. Springer Science+Business Media: 2010; pp 9-23.

48. Kazanietz, M. G., *Mol. Pharmacol.* **2002**, *61*, 759-767.

49. (a) Sonnenburg, E. D.; Gao, T.; Newton, A. C., *J. Biol. Chem.* **2001**, *276*, 45289-45297; (b) Dutil, E. M.; Newton, A. C., *J. Biol. Chem.* **2000**, *275*, 10697-10701.

50. (a) Johnson, J. E.; Giorgione, J.; Newton, A. C., *Biochemistry* **2000**, *39*, 11360-11369; (b) Mosior, M.; McLaughlin, S., *Biophys. J.* **1991**, *60*, 149-159.

51. Gao, T.; Toker, A.; Newton, A. C., *J. Biol. Chem.* **2001**, *276*, 19588-19596.

52. Behn-Krappa, A.; Newton, A. C., *Curr. Biol.* **1999**, *9*, 728-737.

53. Knighton, D. R.; Zheng, J.; Ten Eyck, L. F.; Ashford, V. A.; Xuong, N.-H.; Taylor, S. S.; Sowadski, J. M., *Science* **1991**, *253*, 407-414.

54. Sakai, N.; Sasaki, K.; Ikegaki, N.; Shirai, Y.; Ono, Y.; Saito, N., *J. Cell Biol.* **1997**, *139*, 1465-1476.

55. (a) Oancea, E.; Meyer, T., *Cell* **1998**, *95*, 307-318; (b) Nalefski, E. A.; Newton, A. C., *Biochemistry* **2001**, *40*, 13216-13229; (c) Schaefer, M.; Albrecht, N.; Hofmann, T.; Gudermann, T.; Schultz, G., *FASEB J.* **2001**, *15*, 1634-1636; (d) Toker, A., *Front. Biosci.* **1998**, *3*, D1134-D1147; (e) Newton, A. C., *Chem. Rev.* **2001**, *101*, 2353-2364; (f) Nishizuka, Y., *FASEB J.* **1995**, *9*, 484-496.

56. Gao, T.; Newton, A. C., *J. Biol. Chem.* **2002**, *277*, 31585-31592.

57. (a) Frey, M. R.; Clark, J. A.; Leontieva, O.; Uronis, J. M.; Black, J. D., *J. Cell Biol.* **2000**, *151*, 763-778; (b) Soh, J. W.; Weinstein, I. B., *J. Biol. Chem.* **2003**, *278*, 34709-34716.

58. (a) Tonetti, D. A.; Henning-Chubb, C.; Yamanishi, D. T.; Huberman, E., *J. Biol. Chem.* **1994**, *269*, 23230-23235; (b) Hundle, B.; McMahon, T.; Dadgar, J.; Messing, R. O., *J. Biol. Chem.* **1995**, *270*, 30134-30140.

59. Ivaska, J.; Whelan, R. D. H.; Watson, R.; Parker, P. J., *EMBO J.* **2002**, *21*, 3608-3619.

60. Thelen, M.; Rosen, A.; Nairn, A. C.; Aderem, A., *Nature* **1991**, *351*, 320-322.

61. Hoppe, J.; Hoppe, V.; Schafer, R., *Exp. Cell Res.* **2001**, *266*, 64-73.

62. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G., *Science* **2003**, *300*, 486-489.

63. Alkon, D. L.; Sun, M.-K.; Nelson, T., J., *Trends Pharmacol. Sci.* **2007**, *28*, 51-60.

64. Hecker, E., *Cancer Res.* **1968**, *28*, 2338-2349.

65. Hennings, H.; Boutwell, R. K., *Cancer Res.* **1970**, *30*, 312-320.

66. Driedger, P. E.; Blumberg, P. M., *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 567-571.

67. (a) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y., *J. Biol. Chem.* **1982**, *257*, 7847-7851; (b) Blumberg, P. M.; Jaken, S.; Konig, B.; Sharkey, N. A.; Leach, K. L.; Jeng, A. Y.; Yeh, E., *Biochem. Pharmacol.* **1984**, *33*, 933.

68. (a) Kraft, A. S.; Anderson, W. B.; Cooper, H. L.; Sando, J. J., *J. Biol. Chem.* **1982**, *257*, 13193-13196; (b) Kraft, A. S.; Anderson, W. B., *Nature* **1983**, *301*, 621-623.

69. Furstenberger, G.; Hecker, E., *Planta Med.* **1972**, *22*, 241-266.

70. Szallasi, A.; Krsmanovic, L.; Blumberg, P. M., *Cancer Res.* **1993**, *53*, 2507-2512.

71. Smith, J. B.; Smith, L.; Pettit, G. R., *Biochem. Biophys. Res. Commun.* **1985**, *132*, 939-945.

72. Kraft, A. S.; Smith, J. B.; Berkow, R. L., *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1334-1338.

73. Dell'Aquila, M. L.; Nguyen, H. T.; Herald, C. L.; Pettit, G. R.; Blumberg, P. M., *Cancer Res.* **1987**, *47*, 6006-6009.

74. Pasti, G.; Rivedal, E.; Yuspa, S. H.; Herald, C. L.; Pettit, G. R.; Blumberg, P. M., *Cancer Res.* **1988**, *48*, 447-451.

75. Hennings, H.; Blumberg, P. M.; Pettit, G. R.; Herald, C. L.; Shores, R.; Yuspa, S. H., *Carcinogenesis* **1987**, *8*, 1343-1346.

76. (a) Szallasi, A.; Denning, M. F.; Smith, C. B.; Dlugosz, A. A.; Yuspa, S. H.; Pettit, G. R.; Blumberg, P. M., *Mol. Pharmacol.* **1994**, *46*, 840-850; (b) Szallasi, A.; Smith, C. B.; Pettit, G. R.; Blumberg, P. M., *J. Biol. Chem.* **1994**, *269*, 2118-2124.

77. Choi, S. H.; Hyman, T.; Blumberg, P. M., *Cancer Res.* **2006**, *66*, 7261-7269.

78. Wang, Q. J.; Bhattacharyya, D.; Garfield, S.; Nacro, K.; Marquez, V. E.; Blumberg, P. M., *J. Biol. Chem.* **1999**, *274*, 37233-37239.

79. (a) Hommel, U.; Zurini, M.; Luyten, M., *Nat. Struct. Biol.* **1994**, *1*, 383-387; (b) Xu, R. X.; Pawelczyk, T.; Xia, T. H.; Brown, S. C., *Biochemistry* **1997**, *36*, 10709-10717; (c) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H., *Cell* **1995**, *81*, 917-924.

80. Blumberg, P. M.; Kedei, N.; Lewin, N. E.; Yang, D.; Tao, J.; Telek, A.; Geczy, T., In *Protein Kinase C in Cancer SIgnaling and Therapy*, Kazanietz, M. G., Ed. Springer Science+Business Media: 2010; pp 25-53.

81. Bogi, K.; Lorenzo, P. S.; Szallasi, A.; Acs, P.; Wagner, G. S.; Blumberg, P. M., *Cancer Res.* **1998**, *58*, 1423-1428.

82. (a) Pettit, G. R.; Sengupta, D.; Blumberg, P. M.; Lewin, N. E.; Schmidt, J. M.; Kraft, A. S., *Anti-Cancer Drug Des.* **1992**, *7*, 101-113; (b) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M., *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197-7201.

83. Wender, P. A.; Koehler, K. F.; Sharkey, N. A.; Dell'Aquila, M. L.; Blumberg, P. M., *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4214-4218.

84. Wender, P. A.; Baryza, J. L.; Brenner, S. E.; Clarke, M. O.; Craske, M. L.; Horan, J. C.; Meyer, T., *Curr. Drug Discovery Technol.* **2004**, *1*, 1-11.

85. (a) Wender, P. A.; Irie, K.; Miller, B. L., *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 239-243; (b) Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U., *J. Am. Chem. Soc.* **1998**, *120*, 9159-9167.

86. Schaufelberger, D. E.; Koleck, M. P.; Beutler, J. A.; Vatakis, A. M.; Alvarado, A. B.; Andrews, P.; Marzo, L. V.; Muschik, G. M.; Roach, J.; Ross, J. T.; Lebherz, W. B.; Reeves, M. P.; Eberwein, R. M.; Rodgers, L. I.; Testerman, R. P.; Snader, K. M.; Forenza, S., *J. Nat. Prod.* **1991**, *54*, 1265-1270.

87. Sudek, S.; Lopanik, N.; Waggoner, L. E.; Hildebrand, M.; Anderson, C.; Liu, H.; Patel, A.; Sherman, D. H.; Haygood, M. G., *J. Nat. Prod.* **2007**, *70*, 67.

88. Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S., *J. Am. Chem. Soc.* **1990**, *112*, 7407-7408.

89. Evans, D. A.; Carter, P. H.; Carreira, E. M.; Charette, A. B.; Prunet, J. A.; Lautens, M., *J. Am. Chem. Soc.* **1999**, *121*, 7540-7552.

90. Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S., *Angew. Chem., Int. Ed.* **2000**, *39*, 2290-2294.

91. Trost, B. M.; Dong, G., *Nature* **2008**, *456*, 485-488.

92. Manaviazar, S.; Frigerio, M.; Bhatia, G. S.; Hummersone, M. G.; Aliev, A. E.; Hale, K. J., *Org. Lett.* **2006**, *8*, 4477-4480.

93. Trost, B. M.; Dong, G., *J. Am. Chem. Soc.* **2010**, *132*, 16403-16416.

94. Green, A. P.; Lee, A. T. L.; Thomas, E. J., *Chem. Commun.* **2011**, *47*, 7200-7202.

95. Keck, G. E.; Poudel, Y. B.; Cummins, T. J.; Rudra, A.; Covel, J. A., *J. Am. Chem. Soc.* **2011**, *133*, 744.

96. (a) Wender, P. A.; Schrier, A., *J. Am. Chem. Soc.* **2011**, *133*, 9228; (b) Lu, Y.; Woo, S. K.; Krische, M. J., *J. Am. Chem. Soc.* **2011**, *133*, 13876.

97. Keck, G. E.; Covel, J. A.; Schiff, T.; Yu, T., *Org. Lett.* **2002**, *4*, 1189-1192.

98. Yu, C.-M.; Lee, J.-Y.; So, B.; Hong, J., *Angew. Chem., Int. Ed.* **2002**, *41*, 161-163.

99. (a) Marko, I. E.; Bayston, D. J., *Tetrahedron Lett.* **1993**, *34*, 6595-6598; (b) Marko, I. E.; Mekhalfia, A., *Tetrahedron Lett.* **1992**, *33*, 1799-1802.

100. Covel, J. A. Dissertation Thesis, University of Utah, 2006.

101. (a) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *J. Am. Chem. Soc.* **2008**, *130*, 6660-6661; (b) Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 593-596.

102. (a) Keck, G. E.; Truong, A. P., *Org. Lett.* **2005**, *7*, 2153-2156; (b) Keck, G. E.; Li, W.; Kraft, M. B.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 2277-2280; (c) Kraft, M. B. Dissertation Thesis, University of Utah, 2011.

103. Blumberg, P. M.; Pettit, G. R. In *New Leads and Targets in Drug Research*, Munksgaard, Copenhagen, Krogsgaard-Larsen, P.; Christensen, S. B.; Kofod, H., Eds. Munksgaard, Copenhagen, 1992; pp 273-285.

104. Lewin, N. E.; Blumberg, P. M., *Methods Mol. Biol.* **2003**, *233*, 129-156.

105. Vrana, J. A.; Saunders, A. M.; Srikumar, P. C.; Grant, S., *Differentiation* **1998**, *63*, 33-42.

106. Kedei, N.; Telek, A.; Czap, A.; Lubart, E. S.; Czifra, G.; Yang, D.; Chen, J.; Morrison, T.; Goldsmith, P. K.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Li, W.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2011**, *81*, 1296-1308.

107. Kedei, N.; Lubart, E. S.; Lewin, N. E.; Telek, A.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Keck, G. E.; Kolusheva, S.; Jelinek, R.; Blumberg, P. M., *ChemBioChem* **2011**, *12*, 1242-1251.

108. Kedei, N.; Telek, A.; Michalowski, A. M.; Kraft, M. B.; Li, W.; Poudel, Y. B.; Rudra, A.; Petersen, M. E.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2013**, *85*, 313-324.

109. Kimura, K.; Mizutani, M. Y.; Tomioka, N.; Endo, Y.; Shudo, K.; Itai, A., *Chem. Pharm. Bull.* **1999**, *47*, 1134-1137.

110. Kamano, Y.; Zhang, H.; Morita, H.; Itokawa, H.; Shirota, O.; Pettit, G. R.; Herald, D. L.; Herald, C. L., *Tetrahedron* **1996**, *52*, 2369-2376.

111. (a) Keck, G. E.; Welch, D. S.; Poudel, Y. B., *Tetrahedron Lett.* **2006**, *47*, 8267-8270; (b) Keck, G. E.; Welch, D. S.; Paige, V. K., *Org. Lett.* **2006**, *8*, 3667-3670.

112. (a) Mancuso, A. J.; Swern, D., *Synthesis* **1981**, 165-185; (b) Omura, K.; Swern, D., *Tetrahedron* **1978**, *34*, 1651-1660; (c) Mancuso, A. J.; Huang, S.-L.; Swern, D., *J. Org. Chem.* **1978**, *43*, 2480-2482.

113. (a) Keck, G. E.; Tarbet, K. H.; Geraci, L. S., *J. Am. Chem. Soc.* **1993**, *115*, 8467-8468; (b) Keck, G. E.; Geraci, L. S., *Tetrahedron Lett.* **1993**, *34*, 7827-7828; (c) Keck, G. E.; Krishnamurthy, D.; Grier, M. C., *J. Org. Chem.* **1993**, *58*, 6543-6544; (d) Keck, G. E.; Krishnamurthy, D.; Roush, W. R.; Reilly, M. L., *Org. Synth.* **1998**, *75*, 12-18; (e) Keck, G. E.; Krishnamurthy, D., *J. Am. Chem. Soc.* **1995**, *117*, 2363-2364; (f) Heumann, L. V.; Keck, G. E., *Org. Lett.* **2007**, *9*, 4275-4278.

114. Parikh, J. R.; Doering, W. V. E., *J. Am. Chem. Soc.* **1967**, *89*, 5505-5507.

115. Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I., *Acc. Chem. Res.* **1998**, *31*, 9-17.

116. (a) Keck, G. E.; Castellino, S., *J. Am. Chem. Soc.* **1986**, *108*, 3847-3849; (b) Keck, G. E.; Castellino, S.; Wiley, M. R., *J. Org. Chem.* **1986**, *51*, 5478-5480.

117. (a) Blass, B. E.; Harris, C. L.; Portlock, D. E., *Tetrahedron Lett.* **2001**, *42*, 1611-1613; (b) Dias, L. C.; Glacomini, R., *Tetrahedron Lett.* **1998**, *39*, 5343-5346; (c) Keck, G. E.; Castellino, S., *Tetrahedron Lett.* **1987**, *28*, 281-284.

118. VanRheenen, V.; Kelly, R. C.; Cha, D. Y., *Tetrahedron Lett.* **1976**, *17*, 1973-1976.

119. (a) Doering, W. V. E.; Haines, M. R., *J. Am. Chem. Soc.* **1954**, *76*, 482-486; (b) Fish, P. V.; Johnson, W. S., *J. Org. Chem.* **1994**, *59*, 2324-2335.

120. Evans, D. A.; Allison, B. A.; Yang, M. G.; Masse, C. E., *J. Am. Chem. Soc.* **2001**, *123*, 10840-10852.

121. Oikawa, Y.; Tanaka, T.; Horita, K.; Yonemitsu, O., *Tetrahedron Lett.* **1984**, *25*, 5397-5399.

122. Crouch, D. R., *Tetrahedron* **2004**, *60*, 5833-5871.

123. (a) Keck, G. E.; Truong, A. P., *Org. Lett.* **2005**, *7*, 2149-2152; (b) Truong, A. P. Dissertation Thesis, University of Utah, 2005.

124. Poudel, Y. B. Dissertation Thesis, University of Utah, 2011.

125. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D., *J. Chem. Soc., Chem. Commun.* **1987**, 1625-1627.

126. (a) Tanaka, K.; Ohta, Y.; Fuji, K.; Taga, T., *Tetrahedron Lett.* **1993**, *34*, 4071-4074; (b) Tanaka, K.; Otsubo, K.; Fuji, K., *Tetrahedron Lett.* **1996**, *37*, 3735-3738.

127. (a) Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Peach, M. L.; Blumberg, P. M., *Angew. Chem., Int. Ed.* **2010**, *49*, 4580-4584; (b) Kedei, N.; Lewin, N. E.; Geczy, T.; Selezneva, J.; Braun, D. C.; Chen, J.; Herrmann, M. A.; Heldman, M. R.; Lim, L.; Mannan, P.; Garfield, S. H.; Poudel, Y. B.; Cummins, T. J.; Rudra, A.; Blumberg, P. M.; Keck, G. E., *ACS Chem. Biol.* **2013**, *8*, 767-777.

128. Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4084-4088.

129. Iyer, K.; Rainier, J. D., *J. Am. Chem. Soc.* **2007**, *129*, 12604-12605.

130. Takai, K.; Kakiuchi, T.; Kataoka, Y.; Utimoto, K., *J. Org. Chem.* **1994**, *59*, 2668-2670.

131. Armarego, W. L. H.; Perrin, D. D., *Purification of Laboratory Chemicals*. 4 ed.; Butterworth-Heinemann: Oxford: 1997.

132. Watson, S. C.; Eastham, J. F., *J. Organomet. Chem.* **1967**, *9*, 165-168.

CHAPTER 2

STUDIES ON THE EFFECT OF POLAR FUNCTIONAL GROUPS ON THE NORTHERN HEMISPHERE OF BRYOSTATIN 1 ANALOGUES

Introduction

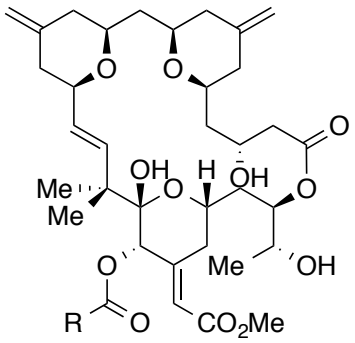
Bryostatin 1, a marine natural product isolated from the bryozoans *Bugula neritina*, has been in the forefront of research studies on promising drugs against various ailments like cancer, Alzheimer's disease, HIV, etc.[1] The unique biological profile of bryostatin 1 has been traced back to its capability to bind with the cysteine-rich C1 domains of PKC isozymes with high affinity.[2] However, various other ligands such as the tumor promoting phorbol esters also bind to PKC isozymes at the same C1 domain but shows opposing character in many biological end points to that of bryostatin 1. In the backdrop of this interesting biological observation, we became interested in investigating further the specific structural features of bryostatin 1 that might explain its unique biology. Our group started a project almost a decade earlier, specifically designed to address this issue, and devised synthetic strategies to prepare bryostatin analogues. With the syntheses of Merle 23, 27, 28, 30, and 32, it became clear that the functional groups at C7, C9, C13, and C30 on the northern hemisphere of bryostatin 1 are not individually critical to the unique biology. However, combinations of these groups are critical to keep the right balance in favor of bryostatin-like properties over PMA-like properties.[3]

Lessons learned from our studies led to a new hypothesis that addresses the need to study the roles of the functional groups in greater detail.

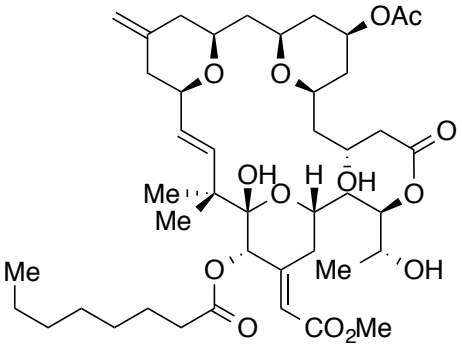
Lessons learned from Keck's analogue program

The first highly potent analogue that came out of our group was Merle 23, which was tested in our collaborator Dr. Peter Blumberg's laboratory at the National Cancer Institute for various biological end points. The experiments conducted by Blumberg and coworkers showed that although Merle 23 bound to PKC with high affinity, it was PMA-alike in attachment and proliferation assays in U937 cells. These two assays were chosen because PMA and bryostatin are known to have different effects for these biological end points in this cell line.[3b, 3e] This was the first demonstrated example of a bryostatin analogue that resembles PMA rather than bryostatin despite its structural similarity with bryostatin 1. This led to further investigations with analogues that differed from bryostatin 1 only in certain positions in the northern hemisphere.

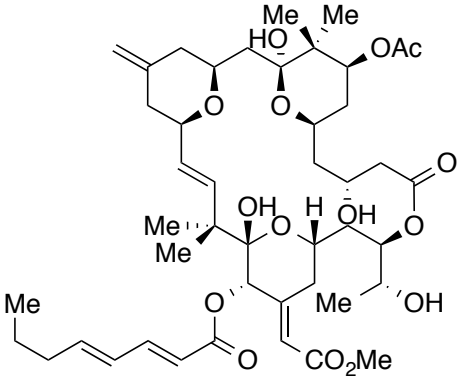
The first attempts with designing analogues focused on utilizing the flagship reaction Keck Yu annulation, commonly known as pyran annulation.[4] The analogue that was first synthesized, i.e., Merle 23, lacked all the polar functional groups in addition to the C8 *gem*-dimethyl group in the northern hemisphere of bryostatin 1 (Figure 2.1). Later, Merle 28 was synthesized that reintroduced all of these functional groups in the A- and B-rings, except for the C30 carbomethoxy group.[3h] The next-generation analogues included Merle 27, Merle 30, and 32. Merle 27 reintroduced the C7 acetate on Merle 23.[3f] Merle 30 excluded the C9 hydroxy group from bryostatin 1 while Merle 32 was structurally similar to Merle 23 but included the C8 *gem*-dimethyl group.[3a, 3g] These were



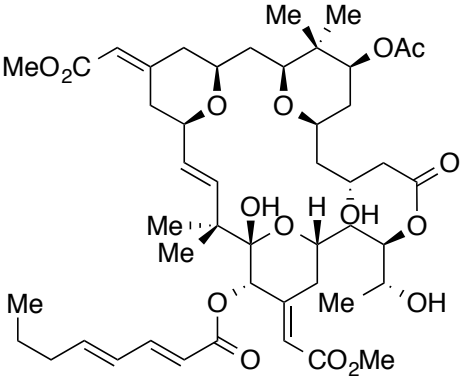
2.1 R = Ph, *Merle 21*
2.2 R = C_7H_{15}, *Merle 22*
2.3 R = C_7H_{11}, *Merle 23*



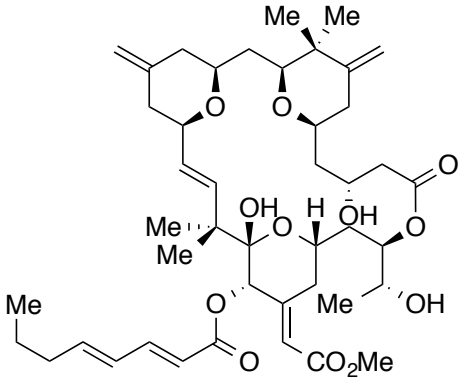
2.4 *Merle 27*



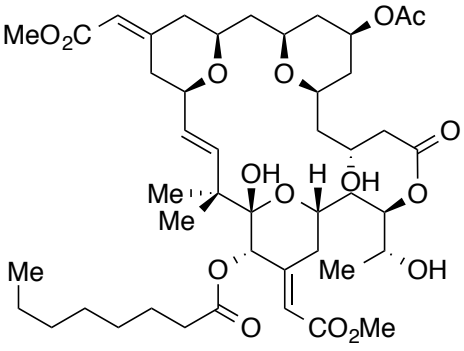
2.5 *Merle 28*



2.6 *Merle 30*



2.7 *Merle 32*



2.8 *Merle 33*

PKCa Binding affinities of analogues

Merle 21, K*i* = 0.70 ± 0.01 nM
Merle 22, K*i* = 1.05 ± 0.04 nM
Merle 23, K*i* = 0.70 ± 0.06 nM
Merle 27, K*i* = 3.00 ± 0.6 nM
Merle 28, K*i* = 0.52 ± 0.06 nM
Merle 30, K*i* = 0.38 nM
Merle 32, K*i* = 1.08 ± 0.16 nM
Merle 33, K*i* = 0.68 nM

Figure 2.1. Various bryostatin analogues from Keck group

assayed for function in the U937 human leukemia cell line, where PMA inhibits proliferation and induces cellular attachment while bryostatin 1 fails to do so and blocks the PMA response.[5] Merle 21-23 were found to show striking resemblance with PMA rather than bryostatin although Merle 23 differs from bryostatin 1 only in the northern hemisphere at C7, C8, C9, and C30 (Figure 2.2), a region previously regarded as just a "spacer domain". The biological profile of Merle 27 (not shown in the Figure 2.2) and 28 showed that the C7 acetate and the C30 carbomethoxy group are not solely responsible for bryostatin biology. Studies with Merle 30 showed that C9 hydroxy group was also not responsible for the unique biology of bryostatin. Merle 32, on the other hand, showed that the C8 *gem*-dimethyl group did not switch the biological profile of Merle 23 from PMA-like to bryostatin-like. Merle 33, a recent analogue synthesized by Dr. Wei Li, lacked the C8 *gem*-dimethyl of Merle 30 and it showed more biphasic nature in its responses and

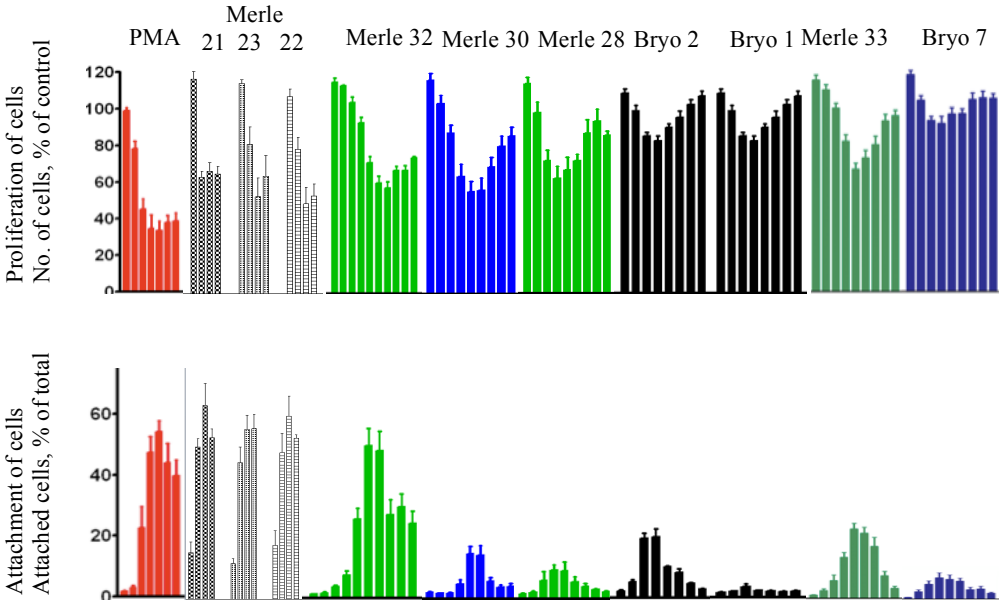


Figure 2.2. Proliferation and attachment assays in U937 cells with various analogues and Bryostatin 1, 2, and 7 under various concentrations[3a, 3d-h]

resembled bryostatin 1.

Various bryostatin analogues were also tested in the Blumberg laboratory in the LNCaP human prostate cancer cells for their induction of 6 of the phorbol ester regulated genes. We found that the degree of response of the LNCaP cells at 8 h to the various derivatives ranged between that of PMA and that of bryostatin 1 or a little below. Similar observations were made in the U937 cells that were treated for 24 h (Figure 2.3). We observed a strong correlation between the bryostatin-like patterns of gene expression and the bryostatin-like patterns of biological response in the U937 cells.[3d]

Among the genes examined in this study, TNFα is worth discussing further as the TNFα protein is an important contributor to the inhibition of LNCaP cell growth in response to phorbol esters. The analogues were intermediate between PMA and bryostatins in their induction of TNFα mRNA expression at 8 h.[3d]

Additionally, time dependence of the modulation of mRNA levels of 8 specific genes was observed and bryostatin 1 showed a markedly transient duration of action (Figure 2.3). The close similarity in the levels of gene induction by PMA and bryostatin 1 at 2 h in the LNCaP cells showed that the induction of different gene sets is not the predominant feature. However, after 2 h, the relative action of bryostatin rapidly diminished. The fact that Merle 23 resembled PMA more closely than bryostatin 1 after 2 h emphasizes the idea that the substituents on the A and B-rings can modulate the duration of action of bryostatin 1.[3d]

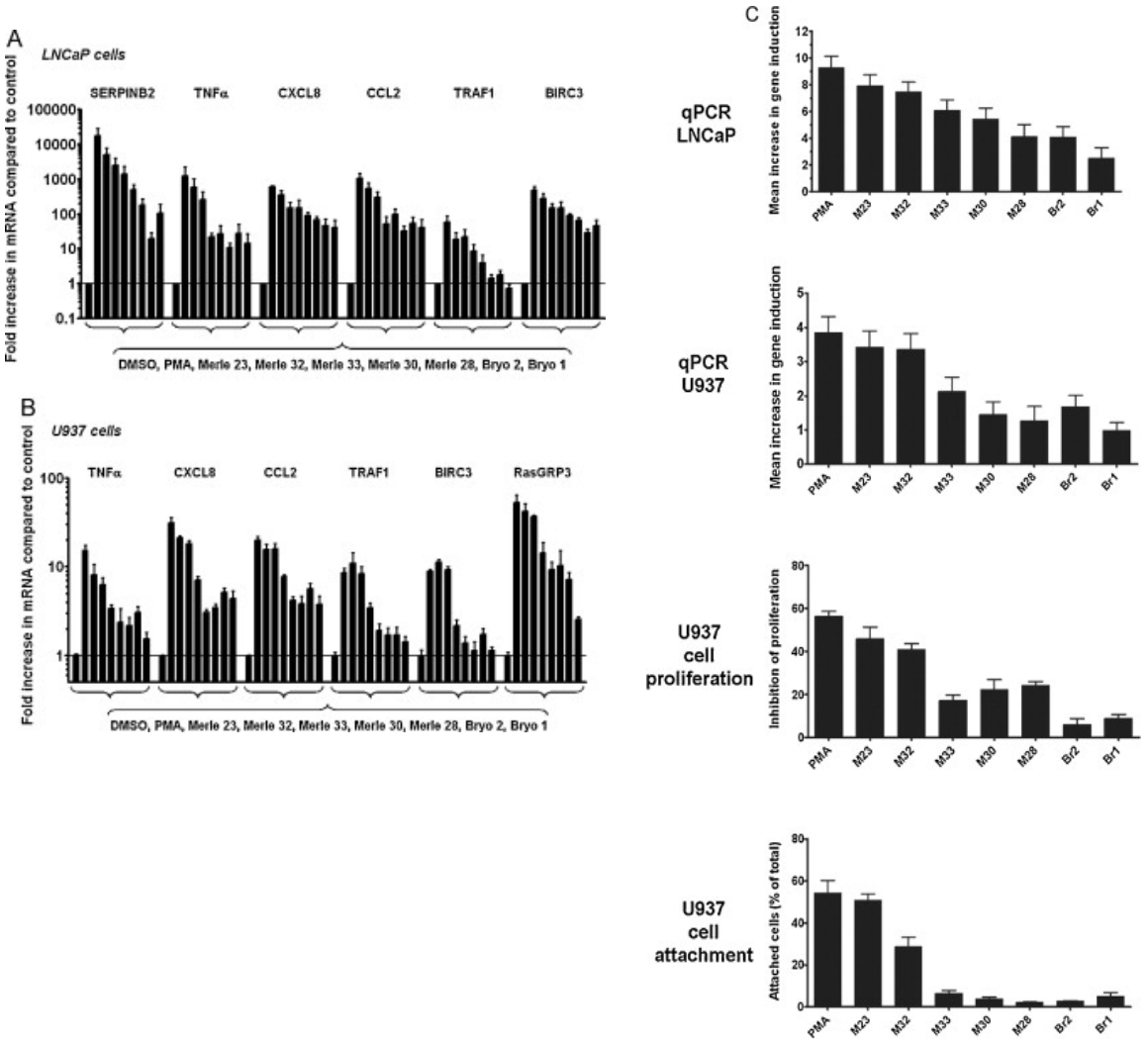


Figure 2.3. Transcriptional activity of different bryostatin analogues

(A) qPCR analysis was performed for the genes on RNA prepared from LNCaP cells treated for 8 h with 1000 nM of the analogues (B) qPCR analysis was performed for the indicated genes on RNA prepared from U937 cells treated for 24 h with 1000 nM of the indicated compounds (100 nM for bryostatin 2) (C) The averaged effect of bryostatin analogues on gene expression in LNCaP and U937 cells and their biological effects in U937 cells.

Polarity hypothesis in relation to the biological properties of bryostatins

With the studies of the analogues synthesized in our group, it became clear that the analogues falls somewhere in between PMA and bryostatin 1 in terms of biology depending on the combination of the hydrophilic and hydrophobic groups. We turned our focus on figuring out the crucial combination that would be essential for bryostatin biology. Bryostatin binds to PKC at the exact same position where phorbol esters bind. Most likely, bryostatin binds to the hydrophobic cleft formed by the pulled apart strands of a β-sheet at the top of C1 domain and this hydrophobic surface is covered by the northern hemisphere of bryostatin 1 upon binding.[6] Additionally, the actual binding of bryostatin 1 with the C1 domain is measured in the presence of phospholipid, which forms the ternary complex of ligand – C1 domain – lipid. Therefore, the complete understanding of bryostatin biology is not possible without considering the critical interactions of the hydrophobic and polar groups of the northern hemisphere of bryostatins with the top surface of the hydrophobic cleft of the C1 domain and the phospholipid. Interestingly, Blumberg group observed that tumor inhibitors or non-promoters such as less lipophilic bryostatin 1 bound to both C1A and C1B domains whereas tumor promoter and more lipophilic PMA bound to C1B domain selectively.[7] Consequently, a further investigation on the polarity of the analogues became crucial at this stage.

Our collaborator Dr. Megan Peach at the Chemical Biology laboratory of the National Cancer Institute performed modeling studies and concluded that the A and B-ring regions of the analogues with PMA-like character were less hydrophilic than the same regions of analogues having bryostatin-like character. Merle 23 and Merle 32 (not

shown in Figure 2.4) has less hydrophilic A and B-ring regions and behaves like PMA. Merle 28 has relatively more hydrophilic groups such as the C9 hydroxy and C7 acetate groups and behaves more like bryostatin than PMA. Merle 30, lacking only the C9 hydroxy group, clearly behaved more like bryostatin than PMA. These correlations prompted us to hypothesize that an appropriate amount of hydrophilicity in the A and B-ring regions along with the bryostatin core structure is needed for the unique bryostatin-like activity and the specific surface formed at the top of C1 domain may define the localization and the protein-protein interactions of the activated PKCs, rendering them with different biological functions.

To examine this hypothesis, we simply calculated clogP values for various "top half" structures (Figure 2.5), with the idea that the southern hemisphere is largely buried in the cleft of the C1 domain and unavailable for interaction with other structures. We envisioned that various combinations of ester and enoate functionalities would match the

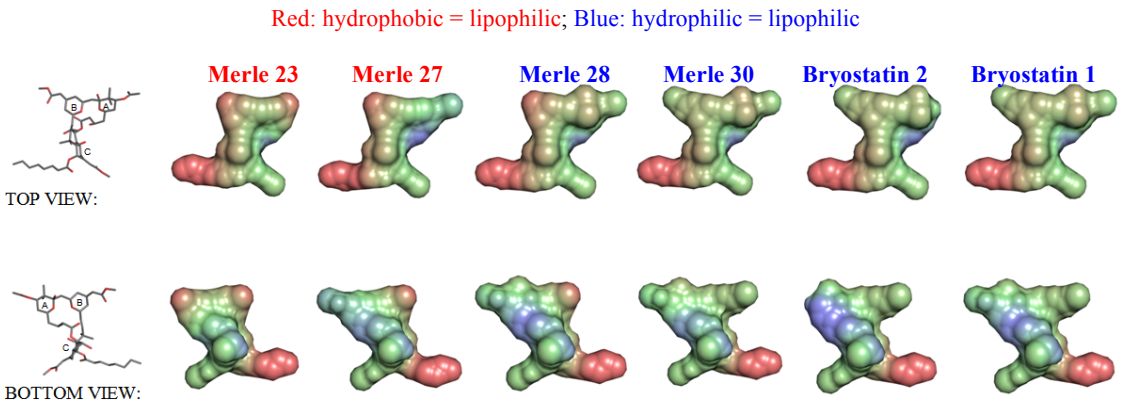


Figure 2.4. Modeling studies by Dr. Megan Peach for Merle 23, 27, 28, 30, bryostatin 2, and 1 (Red indicates less hydrophilic regions and the blue indicates more hydrophilic regions)[8]

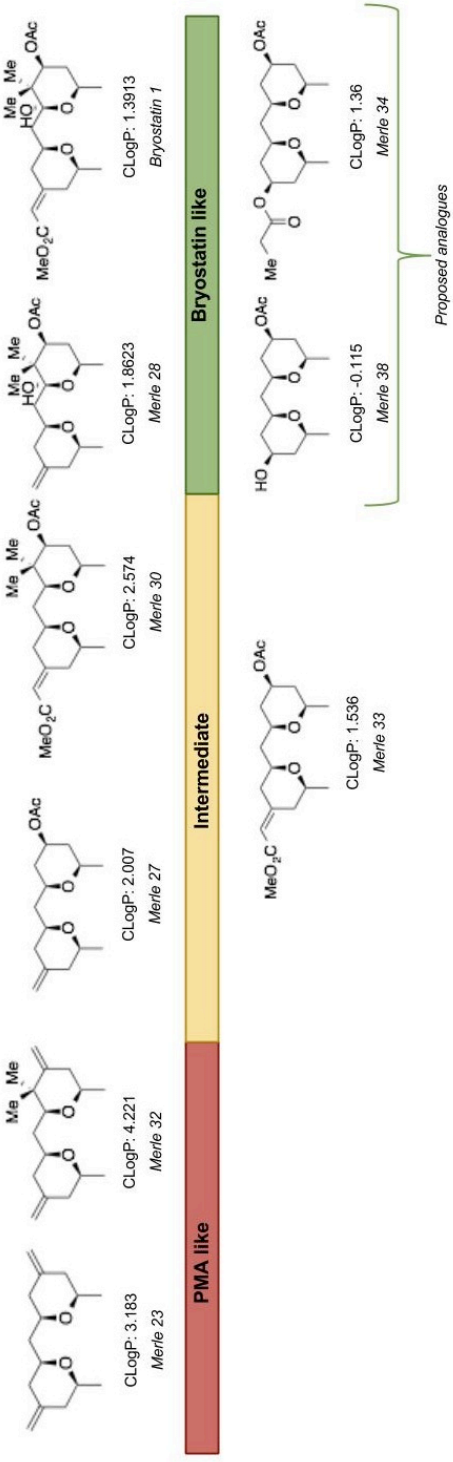


Figure 2.5. Various bryostatin analogues and their corresponding clogP values of the top halves

calculated clogP values. Intriguingly, we found a trend between polarity and corresponding biological responses of the analogues. Although the analogues which fall under the intermediate category do not quite follow the trend, we hoped to get more data points by synthesizing more analogues to validate this hypothesis. In line with this hypothesis, we proposed two possible analogues (Merle 34 and 38 in Figure 2.5). Merle 34 (**2.9**) very closely resembles of the top half of bryostatin 1 in clogP value while Merle 38 (**2.10**) has a very hydrophilic top half. The propionate group at C13 of Merle 34 was chosen to closely match clogP value for the "top half" of the structure to that of bryostatin 1. A shorter chain (acetate) or a longer chain (butanoate) would force the value of clogP for the analogue to deviate further away from the corresponding number for bryostatin 1.

Retrosynthetic analysis of Merle 34 and 38

The retrosynthetic analysis of the analogues Merle 34 and 38 is outlined in Figure 2.6. Using our experience from the syntheses of bryostatin 1 and 7, we envisaged that a similar disconnection at the C1 ester bond and at the B-ring pyran across C15 would provide us with the two equally complex synthetic equivalents of a β-hydroxyallyl silane **2.12** and the fully functionalized C-ring aldehyde **2.11**. Our flagship reaction of pyran annulation would couple the two complex intermediates to form the tricyclic core of Merle 34 and 37. After some protecting group manipulation and several oxidation reactions, we should be able to macrolactonize the tricyclic core at C1.

The A-ring β-hydroxyallyl silane **2.12** could be prepared from an ester **2.13** using Bunelle reaction using trimethylsilylmethyl Grignard reagent (Figure 2.7). The

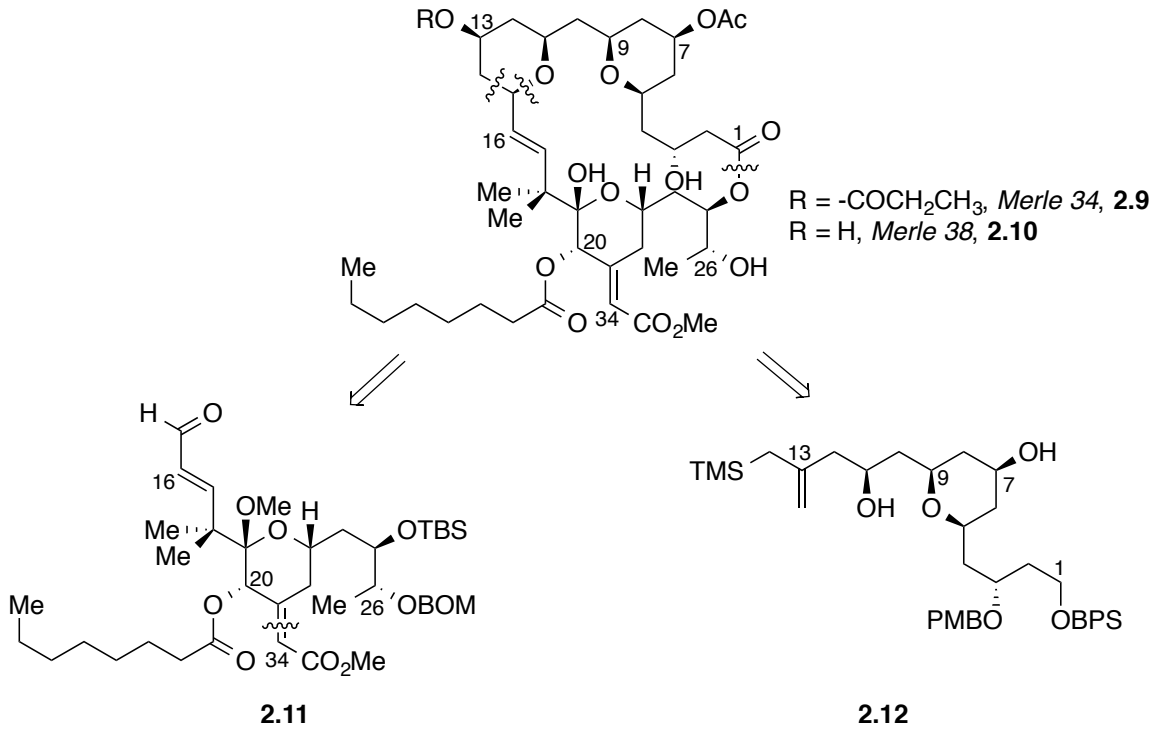


Figure 2.6. Retrosynthetic analysis of Merle 34 and 38

stereocenter at C7 could rise from a diastereoselective reduction of the corresponding ketone of the olefin **2.14**. The A-ring substituted pyran **2.14** could arise from another pyran annulation between the aldehyde **2.15** and β-hydroxyallyl silane **2.17**. The stereocenter at C11 could be installed by a Noyori asymmetric reduction of the β-keto ester **2.16**. The β-hydroxyallyl silane **2.17** was disconnected to form the known synthetic equivalents of stannane **2.18** and the aldehyde **2.19**. A chelation-controlled diastereoselective allylation would produce the β-hydroxyallyl silane **2.17**. The C3 stereocenter on the aldehyde **2.19** could be installed using a catalytic asymmetric allylation (CAA) using (*R*)-BITIP catalyst on the corresponding aldehyde at C3.

The fully functionalized C-ring aldehyde **2.11** (Figure 2.8) was disconnected first at the C21 enoate, which could be installed on the ketone **2.20**. The ketone could arise

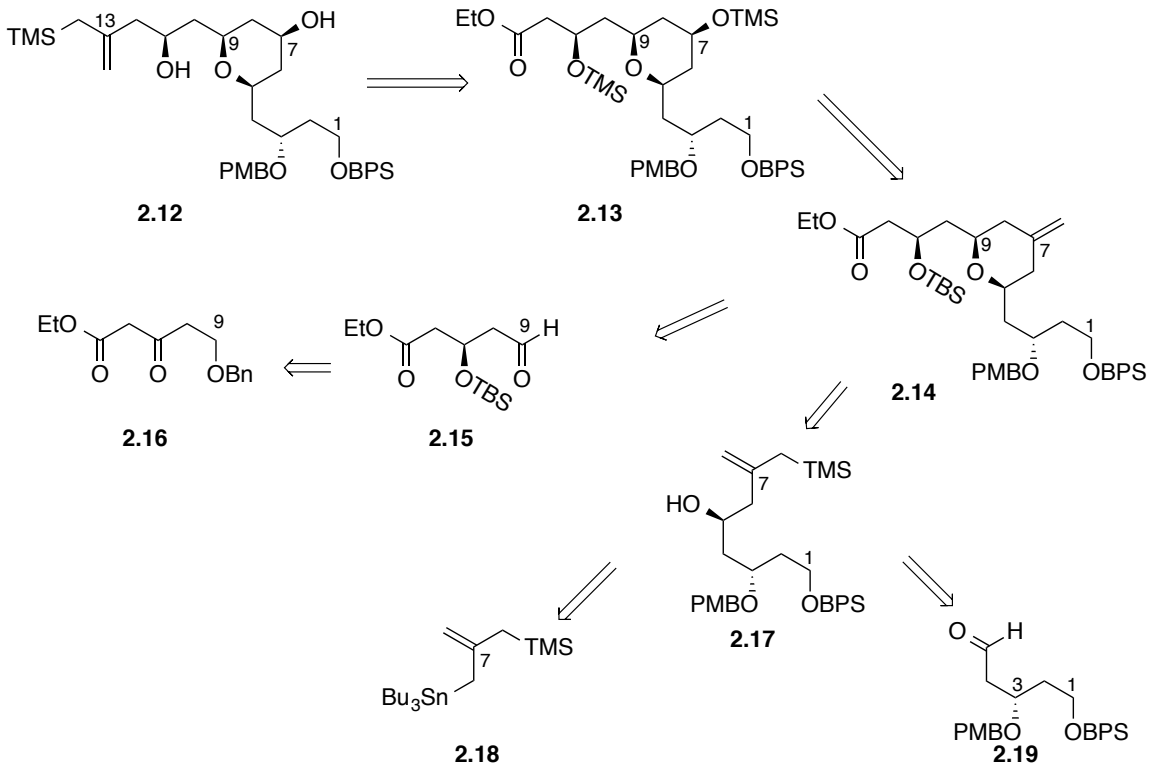


Figure 2.7. Retrosynthetic analysis of A-ring intermediate **2.12**

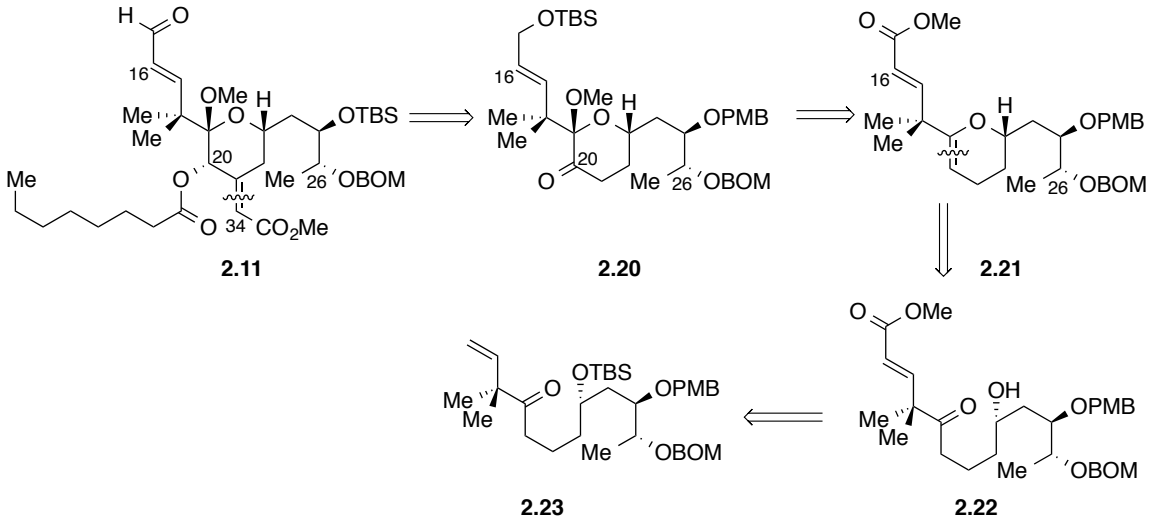


Figure 2.8. Retrosynthetic analysis of C-ring intermediate **2.11**

from the glycal **2.21**. The synthetic precursor of the C15 TBS-protected alcohol could be an α,β-unsaturated methyl ester. The C-ring glycal **2.21** was envisioned to arise from the dehydrative cyclization of the keto-alcohol **2.22**. The keto-alcohol **2.22** could arise from the known intermediate **2.23**. Ozonolysis on the terminal olefin on **2.23** followed by a Horner-Wadsworth-Emmons olefination would provide us with the compound **2.22**.

Synthesis of Merle 34 and 38

Synthesis of the A-ring β-hydroxyallyl silane **2.12**

The synthesis of the A-ring β-hydroxyallyl silane **2.12** began with the monoprotection of the 1,3-propanediol with a robust BPS group (Figure 2.9). Swern oxidation of the free alcohol provided the aldehyde **2.26** in high yield.[9] With the aldehyde

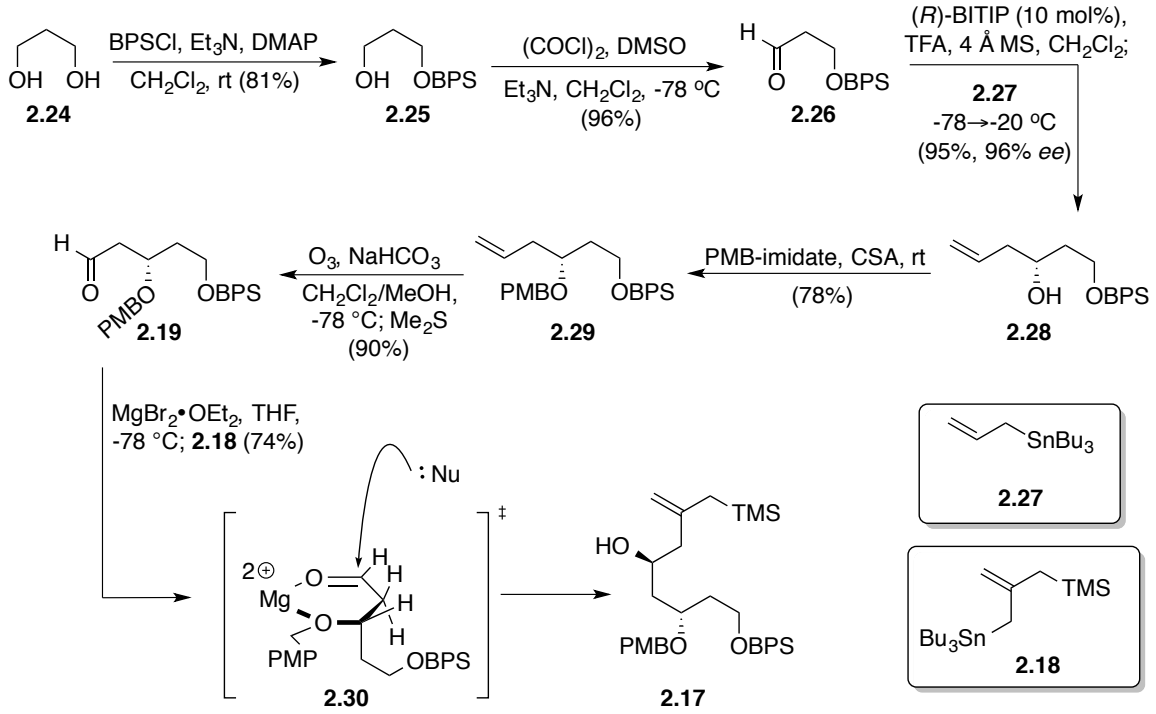


Figure 2.9. Synthesis of the β-hydroxyallyl silane **2.17**

2.26 in hand, we applied Keck asymmetric allylation using (*R*)-BITIP as catalyst to install the C3 stereocenter in high enantioselectivity.[10] Having the first stereocenter installed, we then utilized this stereocenter to install the next stereocenter at C5. The free alcohol was protected as PMB ether **2.29** to utilize the chelating ability of the PMB group. Ozonolysis on the terminal alkene provided the aldehyde **2.19**. Using $MgBr_2 \bullet OEt_2$ as Lewis acid to form the desired chelated transition state **2.30**, allyl silane **2.18** was added to the aldehyde **2.19** to form the β-hydroxyallyl silane **2.17** as a single diastereomer.[11]

Synthesis of the A-ring pyran compound **2.14** was previously achieved by Dr. Wei Li and scaled up by Kevin McGowan from our group. The synthesis of the aldehyde **2.15** began by reacting benzyl alcohol with acrylonitrile under basic conditions to prepare the benzyl ether **2.32** (Figure 2.10). Using Blaise reaction on the ethyl bromoacetate and **2.32** as the electrophile, we were able to prepare β-keto ester **2.16** in multigram scales.[12] With the β-keto ester **2.16** in hand, we utilized Noyori asymmetric hydrogenation to reduce the ketone with excellent enantioselectivity to install the stereocenter at C11.[13] The alcohol was then protected as TBS ether, which was then followed by removal of the benzyl group. Swern oxidation of the free alcohol provided the aldehyde **2.15**. With the two partners **2.17** and **2.15** in hand, we were then able to couple them using the flagship pyran annulation to form the A-ring pyran **2.14**. The next step was to switch the protecting group from TBS to TMS keeping the lability of the TMS group in mind for a later stage manipulation. Tosic acid monohydrate was used at ambient temperature to remove the TBS group in the presence of the BPS group at C1. However, the diol **2.36** was also observed although caution was taken to minimize the deprotection of the BPS

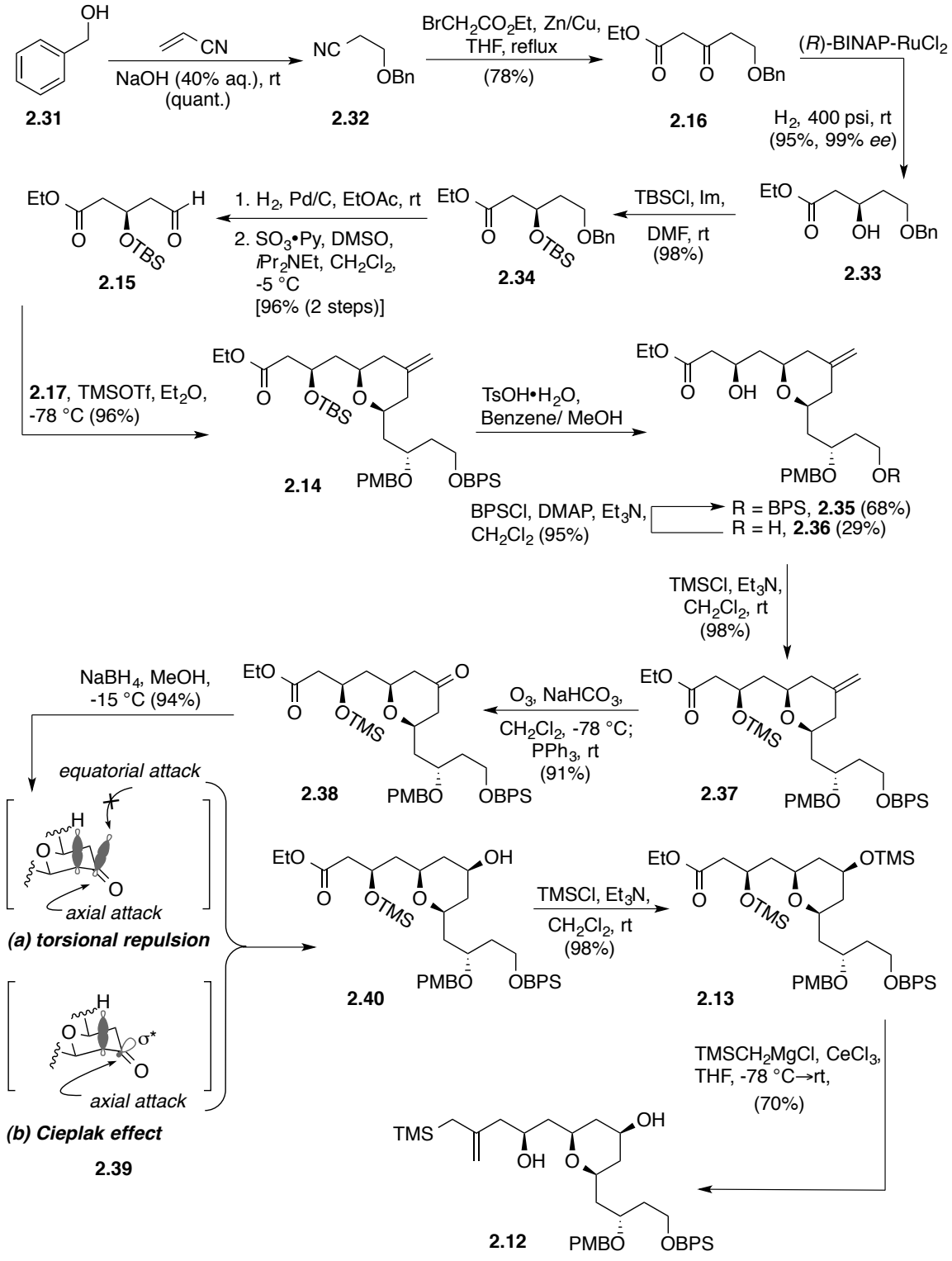


Figure 2.10. Synthesis of the A-ring β-hydroxyallyl silane **2.12**

group. The diol was converted to **2.35** without any issue. The alkene **2.37** was then subjected to ozonolysis followed by $NaBH_4$ reduction to provide the alcohol **2.40** as a single diastereomer. The axial attack of small hydride sources like $NaBH_4$ on cyclohexanone-derived ketone is a well-documented phenomenon and generally explained by the torsional repulsion between the electrons of the forming bond and the vicinal axial C-H bonds in case of equatorial attack and a stabilizing effect (*Cieplak effect*) by the vicinal C-H bonds on the forming σ* orbital in case of axial attack.[14] The free alcohol was then protected temporarily as a TMS ether to form **2.13**. With the ester **2.13** in hand, we applied the Bunelle reaction followed by an acidic work-up to provide the β-hydroxyallyl silane **2.12**.[15] Anhydrous $CeCl_3$ was very essential for this reaction and any trace of moisture could potentially ruin the active reagent.

Synthesis of the aldehyde **2.11**

Synthesis of the ketone **2.23** used a series of 1,2 and 1,3 chelation-controlled allylations; hydroformylation followed by prenylation has been reported earlier from our group and scaled up by Dr. Matthew Kraft.[16] The terminal olefin on **2.23** was cleaved to form the aldehyde, which was then subjected to Horner-Wadsworth-Emmons olefination to provide the α,β-unsaturated methyl ester **2.42**. In old routes we used to install a thioester in presence of the methyl ester. The goal was to reduce the thioester selectively to an aldehyde subsequently. Additionally, we functionalized the C-ring later in the synthesis. In contrast, the newer strategy was to functionalize the C-ring ahead of pyran annulation to make the synthesis more convergent. For this strategy, reduction of the methyl ester fully to an alcohol seemed a prudent choice, as protected alcohols are easier

to manipulate under various reaction conditions. Next, removal of TBS group was followed by dehydrative cyclization providing the C-ring glycal **2.21**. The ester was then reduced to a primary alcohol **2.43**, which was then protected as the TBS ether to give **2.44**. The C-ring glycal was then further functionalized across the C19-C20 unsaturation to form the ketal at C19 and ketone at C20. Thus, epoxidation of the olefin with MMPP followed by methanolysis formed the methyl ketal with an alcohol at C20 as a mixture of diastereomers. This mixture was then immediately oxidized under Ley conditions to the ketone **2.20** as essentially a single diastereomer. With the ketone in hand, we attempted the aldol condensation, which went smoothly without any incident. The exclusive formation of the *E*-enoate is not a very well understood reaction outcome. The initial attack on the aldehyde by the enolate of the ketone **2.20** can proceed via a chair like transition state to form the axially substituted aldol adduct or via a twist boat-like transition state to form the more stable equatorially substituted adduct. The stereochemistry at C21 of the intermediate aldol product could not be determined as the dehydration occurred readily under the reaction condition. The elimination of the C34 hydroxyl group could be an SN^2 type (for axially substituted adduct) or E^1CB type (for equatorially substituted adduct) elimination. For all the possibilities, the reaction outcome suggests that a 1,3 allylic strain between the ketone carbonyl and the methyl ester forces the ester away from the ketone carbonyl in the developing transition state, leading to the condensation product **2.45**. The keto ester **2.45** was later observed to be unstable and was therefore immediately converted to the octanoate **2.48** via Luche reduction and esterification. The Luche reduction was found to form the α-alcohol exclusively, possibly due to the top face attack by the hydride reagent, as explained in **2.46** (Figure 2.11). At

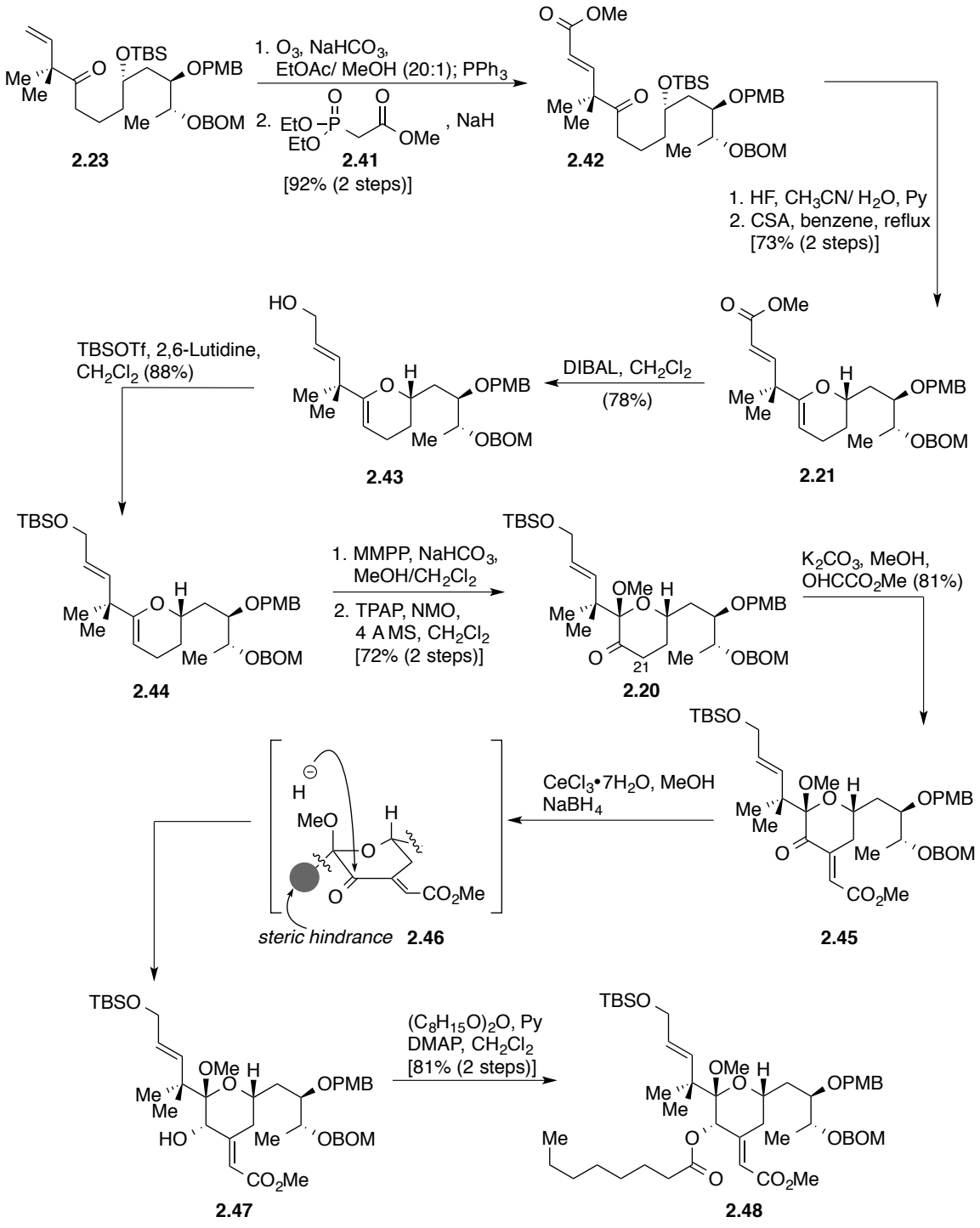


Figure 2.11. Synthesis of fully functionalized C-ring intermediate

this stage, switching the C25 PMB group became necessary as we anticipated potential problems resulting from the lack of chemoselectivity between the C3 PMB ether and the C25 PMB ether in the pyran annulation product.

We decided to switch the C25 PMB with a TBS group. After the removal of the PMB with DDQ, protection of C25 with TBS group and removal of the primary allylic TBS group chemoselectively provided the alcohol **2.51** (Figure 2.12). Dess Martin periodinane oxidation of the allylic alcohol **2.51** provided the required aldehyde **2.11**. *t*BuOH proved to be beneficial in both the steps using DDQ and DMP and resulted in

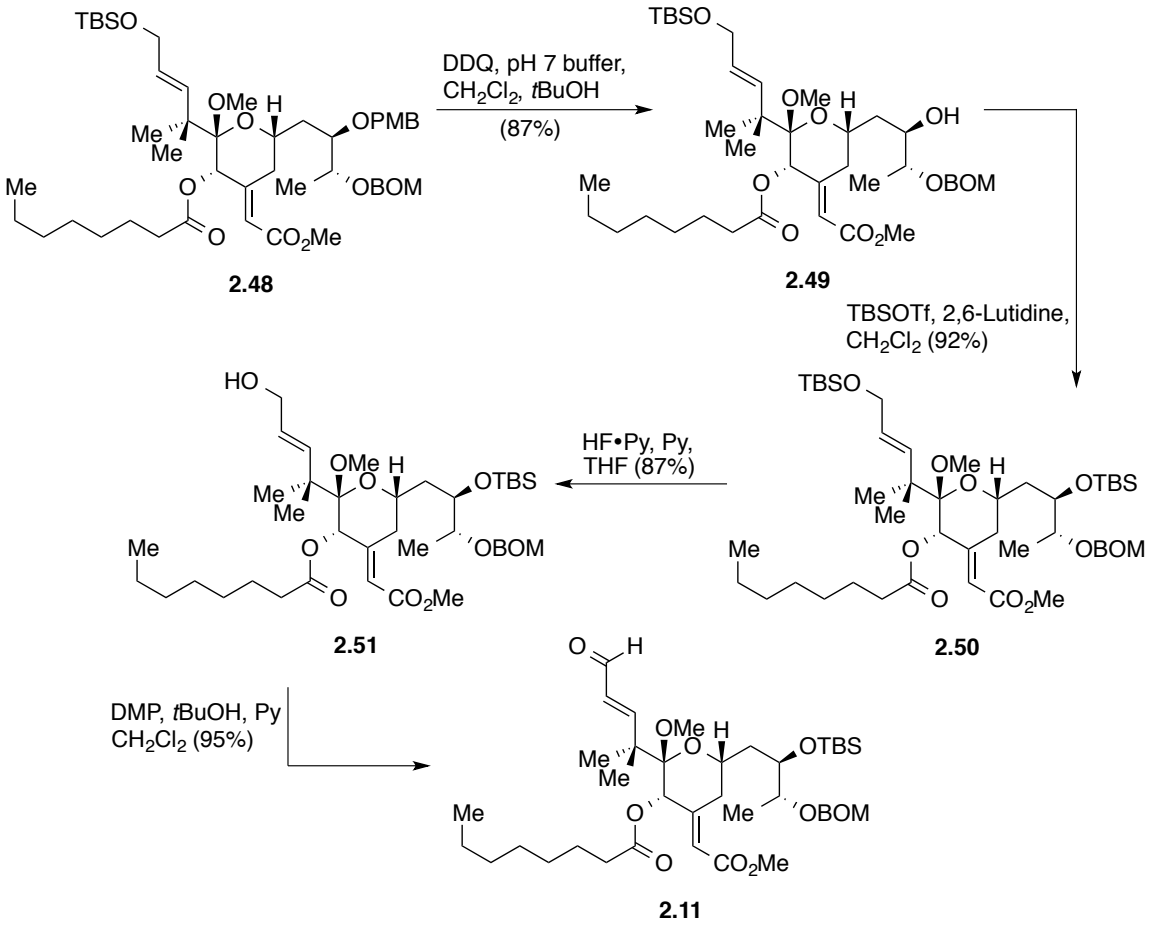


Figure 2.12. Synthesis of the C-ring aldehyde **2.11**

higher yields.

Completion of the syntheses of Merle 34 and 38

With the aldehyde **2.11** and the β-hydroxyallyl silane **2.12** in hand, we then attempted the pyran annulation reaction in the presence of the free alcohol at C7 (Figure 2.13).[4, 16a] This reaction proved to be sensitive to moisture and temperature. The reaction barely went at -78 ^{o}C and went to completion only after raising the temperature to -50 ^{o}C. The side products included elimination of the C20 proton along with the methoxy group at C19. The reaction also proved that the free hydroxyl group at C7 can be tolerated under the pyran annulation conditions. The C7 free hydroxyl group was then converted to the acetate. The C1 BPS group was then targeted for removal using TBAF under acidic conditions to provide the alcohol **2.54**. Notably, we were able to remove the BPS group in the presence of the C25 secondary TBS group. The alcohol **2.54** was then subjected to sequential Parikh-Doering and Lindgren oxidations to provide the carboxylic acid **2.56**.[17] The C25 TBS ether was then removed to form the seco-acid, which was then subjected to Yamaguchi macrolactonization to form **2.57** (Figure 2.14). Careful olefin cleavage at C13-C30 using saturated ozone solution provided the ketone **2.58** in good yield. With the ketone in hand, we then attempted the reduction using small hydride source as $NaBH_4$. We were delighted to observe that the reaction went smoothly to produce the alcohol **2.59** with high diastereoselectivity (7:1). The minor isomer was mixed with other impurities and could not be purified after repeated column chromatography. The major isomer **2.59** was then esterified with propionic anhydride. The stereochemistry of the C13 hydroxy group was established by nOe studies using NMR. With the protected analogue in hand,

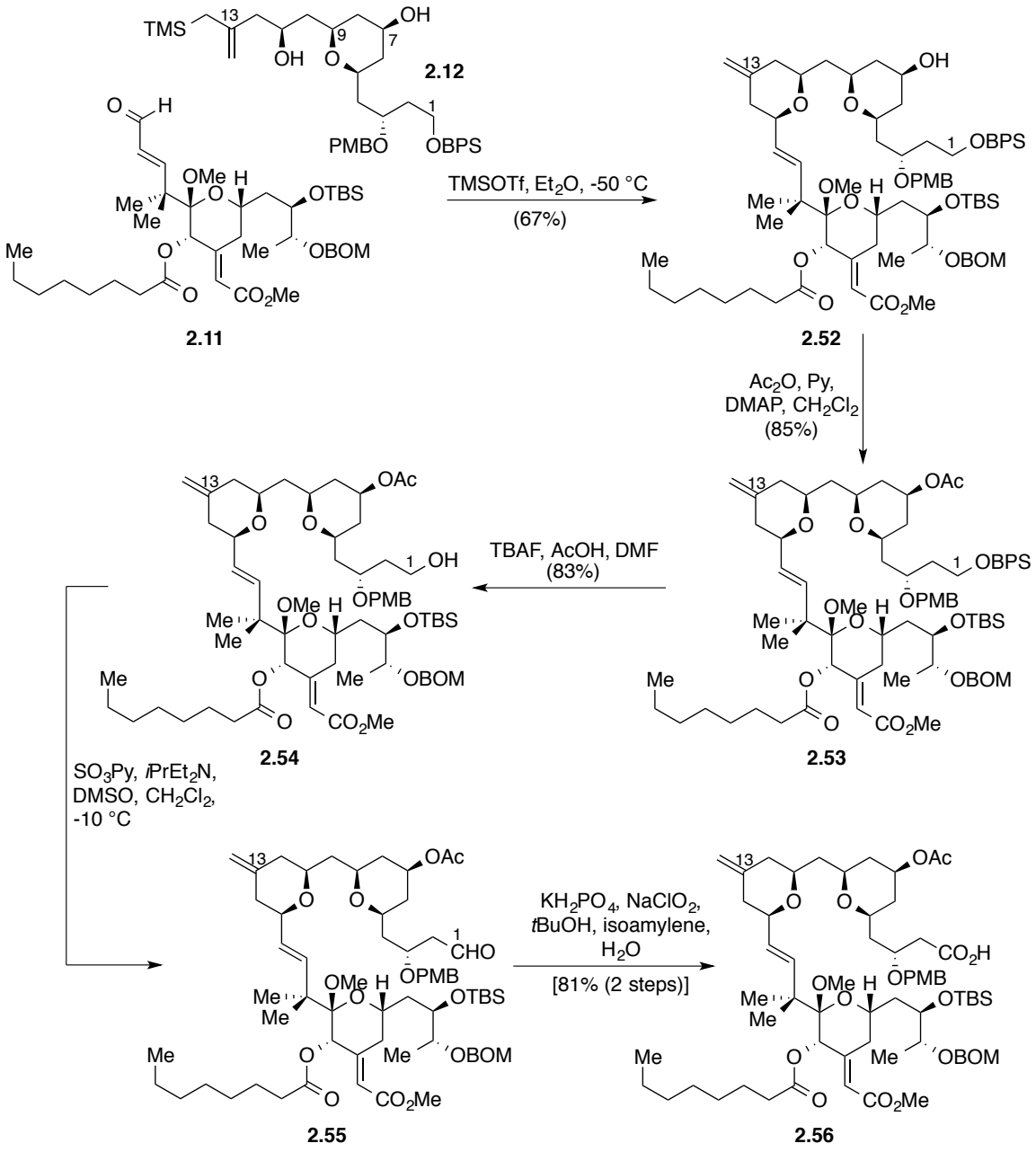


Figure 2.13. Synthesis of the advanced intermediate **2.56**

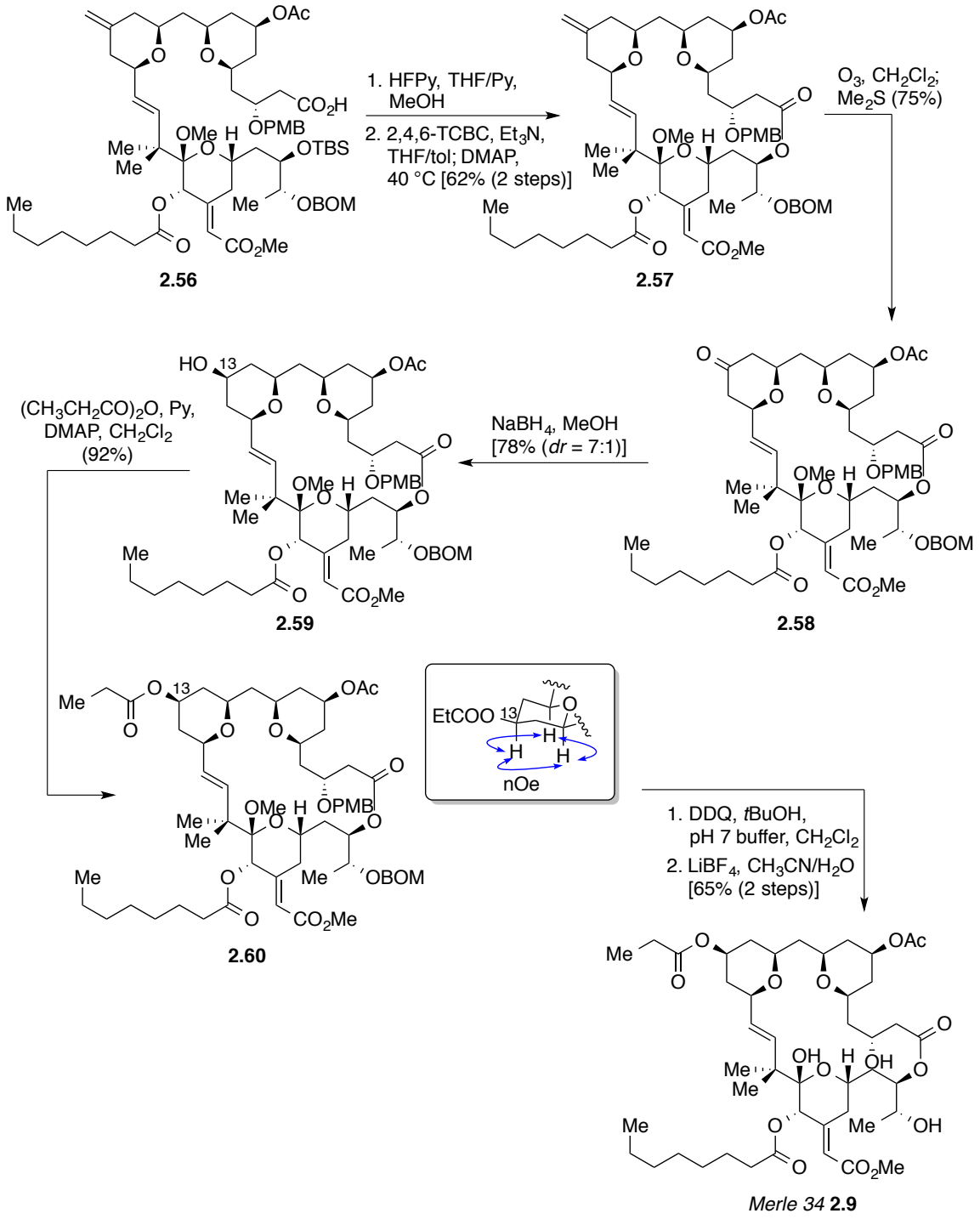


Figure 2.14. Completion of the synthesis of Merle 34

we then removed the PMB group using DDQ. Global deprotection under Lipshutz conditions provided the analogue Merle 34.[18] Interestingly, after 1-2 h, TLC analysis showed the starting material was consumed with the formation of a nonpolar spot above the spot corresponding to the substrate in accord with the previous observations made by Dr. Yam Poudel during the total synthesis of bryostatin 1. This indicates the formation of the intramolecular hydrogen-bonding network observed in the crystal structure between the two pyran oxygen atoms, the C3 hydroxy group, and C19 hemiketal group. After 6 h, the spot moved downwards with much lower R_f value, indicating the removal of the BOM group. The common intermediate **2.59** was then treated with DDQ followed by global deprotection to provide the other analogue Merle 38 (Figure 2.15).

Biological evaluation of Merle 34 and 38

Binding affinity studies

The biological evaluation of Merle 34 and 38 began with finding out the binding affinity of the ligands to mouse PKCα. Interestingly, both ligands were found to bind PKCα with much diminished affinity compared to bryostatin 1. Merle 34 was found to

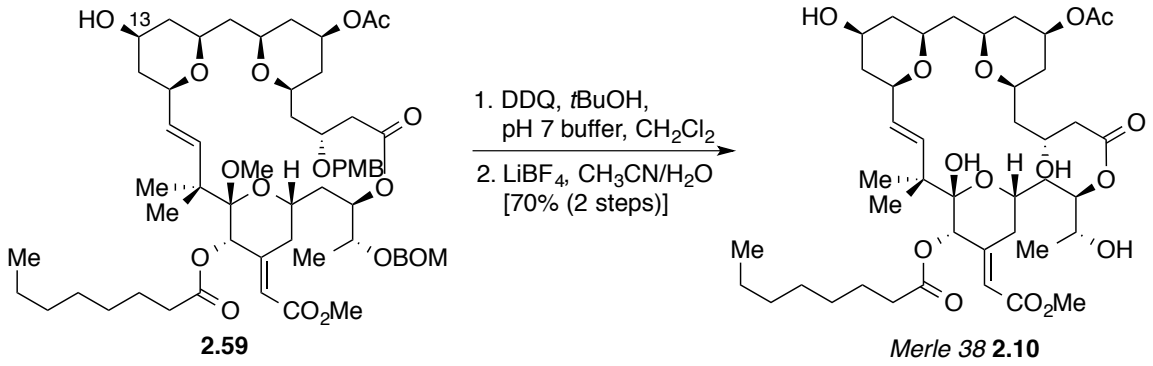


Figure 2.15. Completion of the synthesis of Merle 38

have K*i* = 16.3 nM while Merle 38 had K*i* of 13.21 nM. The decreased potency clearly indicated a potential role of the B-ring alkene functional group at C13-C30 and the C7 acetate in combination with other groups on the northern hemisphere of bryostatin 1. The binding affinity data also suggested that additional interactions with the carbomethoxy enoate at C13-C30 on the northern hemisphere could be responsible for binding of the ligand to PKC, which was also evident from the comparison of the binding affinity data of Merle 27 (K*i* = 3.00 ± 0.06 nM) and Merle 33 (0.68 nM). The nature of the interactions is not known at this point and requires further investigation.

Proliferation and attachment assays on U937

Although Merle 34 "top half" had a comparable clogP value with that of bryostatin 1, the proliferation and attachment assays with U937 cells showed that Merle 34 behaved very similarly to Merle 33. PMA inhibited proliferation while bryostatin 1 had little effect on it and Merle 34 had a biphasic response in this cell line. When used in combination with PMA, Merle 34 was able to reverse the response of PMA in a dose-dependent manner. Similarly in attachment assay, PMA induced attachment while bryostatin 1 had very little effect. Merle 34 again showed a biphasic response in a dose-dependent manner. When applied in combination with PMA, Merle 34 reversed the response of PMA at higher concentrations. Evidently, Merle 34 is intermediate between PMA and bryostatin 1 in these two assays and resembled Merle 33 with a bit more PMA-like character (Figure 2.16).

Merle 38 was calculated to have a highly hydrophilic "top half" with a very low clogP value. In the proliferation assay with U937 cells, we observed that Merle 38

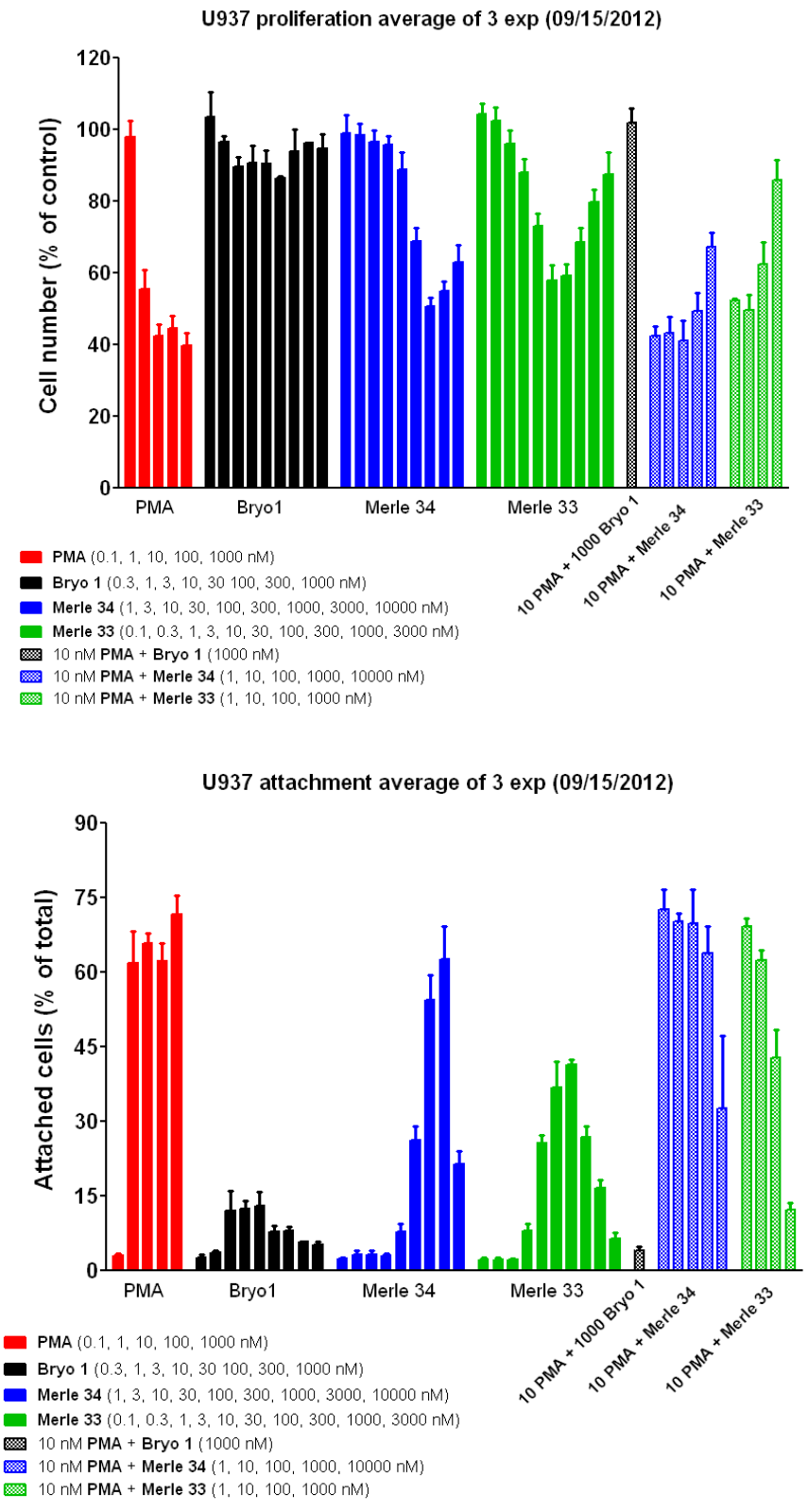


Figure 2.16. Proliferation and attachment assays on U937 cells with Merle 34

modestly inhibited proliferation, but only at high doses. However, the lower potency and thus higher concentrations required with Merle 38 make these comparisons more difficult. When applied in combination with PMA, Merle 38 did not reverse the response to PMA. In the attachment assay, Merle 38 was found to induce attachment albeit to a lesser extent than PMA. When combined with PMA, Merle 38 failed to reverse the responses of PMA (Figure 2.17).

Studies on TNFα secretion in U937 cells

Our collaborator, Dr. Blumberg, also studied the effects of the analogues on TNFα secretion in U937 cells. Interestingly, in this assay, Merle 38 shows a biphasic nature in a dose-dependent manner and approaches bryostatin 1 like properties only at much higher concentrations (Figure 2.18). When used in combination with PMA, Merle 38 reversed the responses of PMA to a considerable extent at higher concentrations. Again, lower potency of Merle 38 makes it harder for direct comparison with PMA and bryostatin 1.

Studies on Toledo cells

Bryostatin 1 and PMA behave very similar in human non-Hodgkin lymphoma cell line known as Toledo cells. Bryostatin 1 (IC_{50} = 0.076 ± 0.00 nM) and PMA (IC_{50} = 0.076 ± 0.17 nM) have almost identical IC_{50} and Merle 38 (IC_{50} = 104.83 ± 2.45 nM) was found to have much less potency than bryostatin 1 (Figure 2.19). In this assay, Merle 38 inhibited proliferation only at higher concentrations and resembled another recent analogue Merle 37 synthesized by graduate student Mr. Mark Peterson from our group.

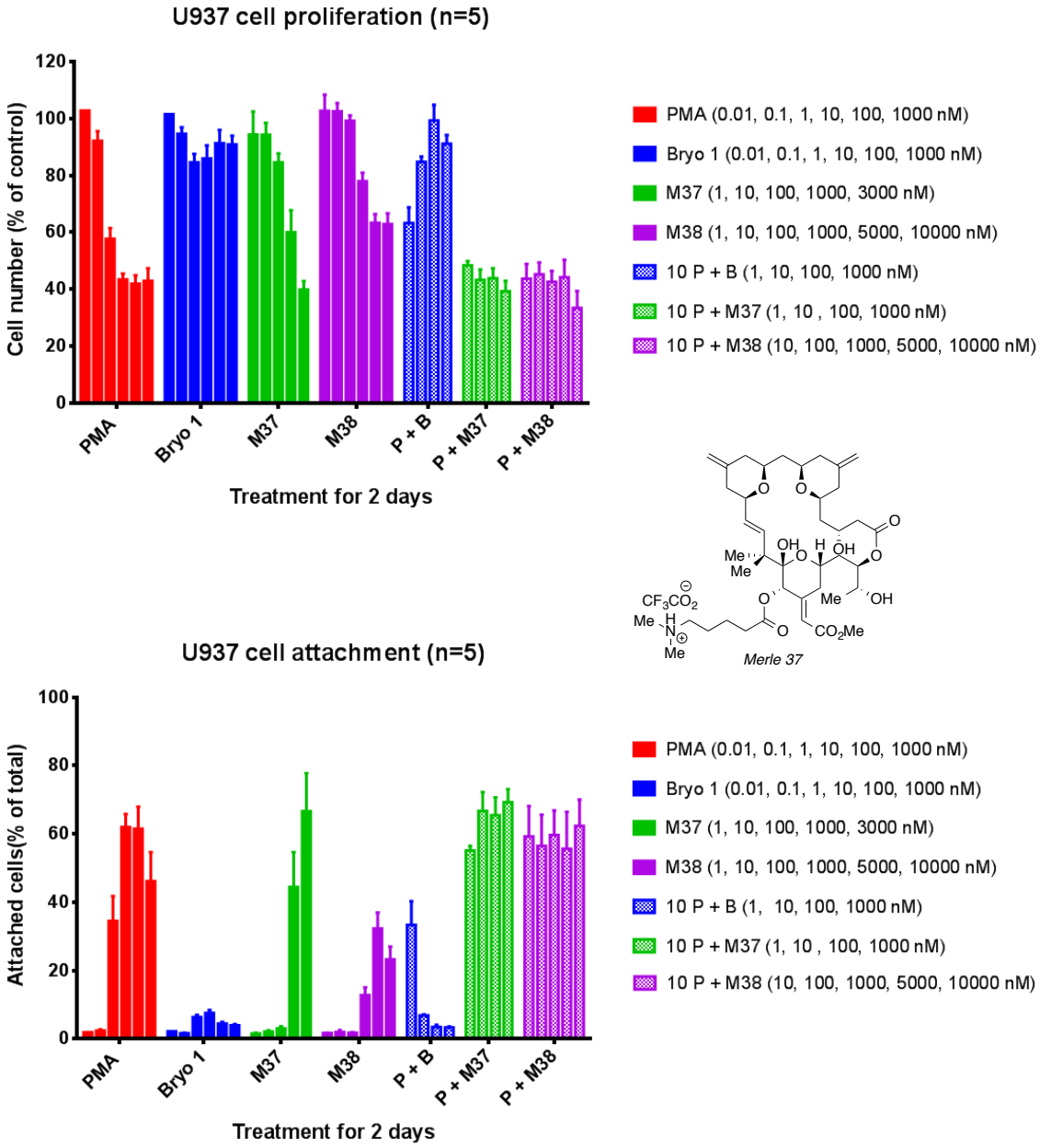


Figure 2.17. Proliferation and attachment assays on U937 cells with Merle 38 and recently synthesized Merle 37

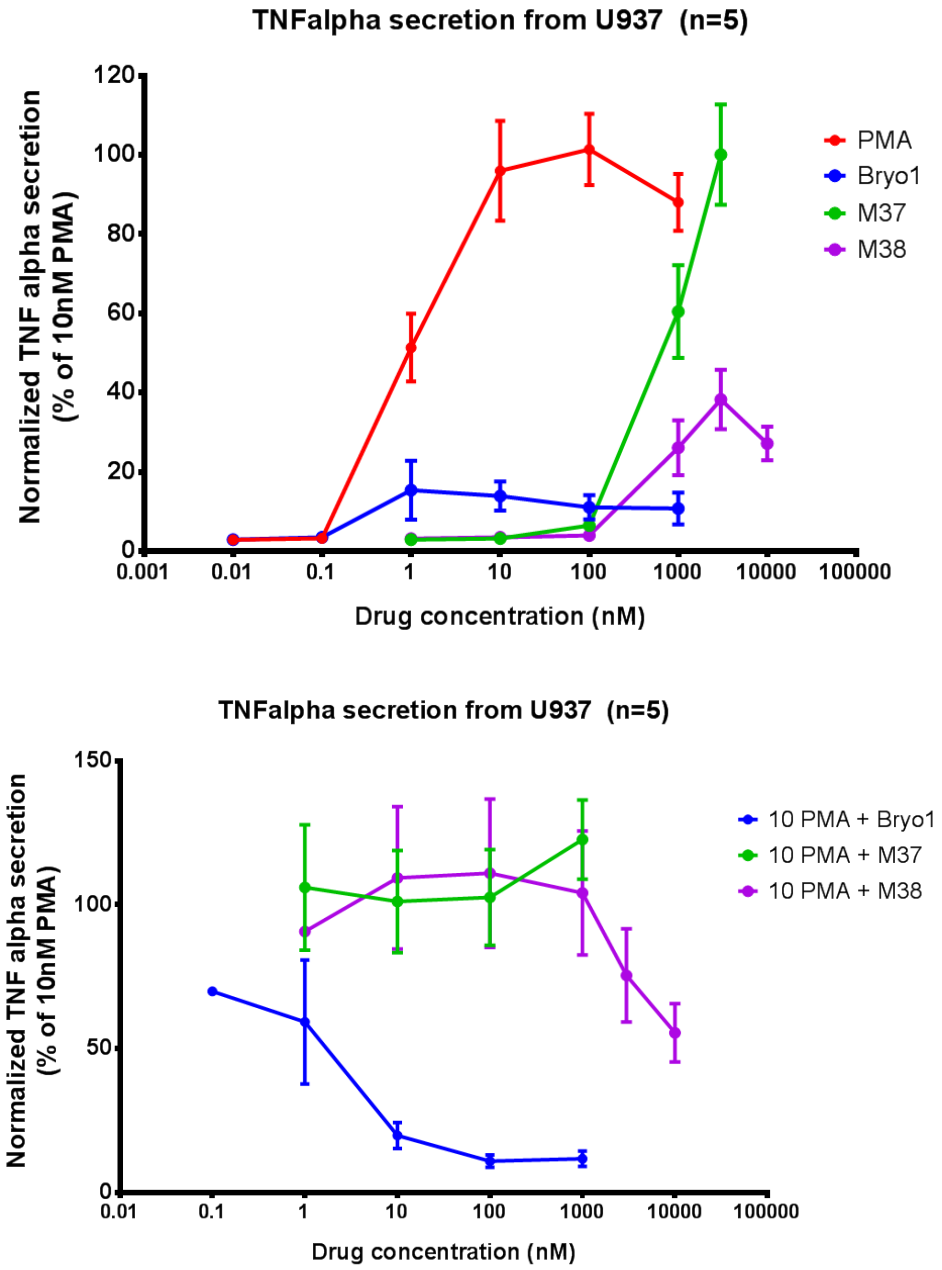


Figure 2.18. TNFα secretion assay on U937 cells with Merle 37 and Merle 38

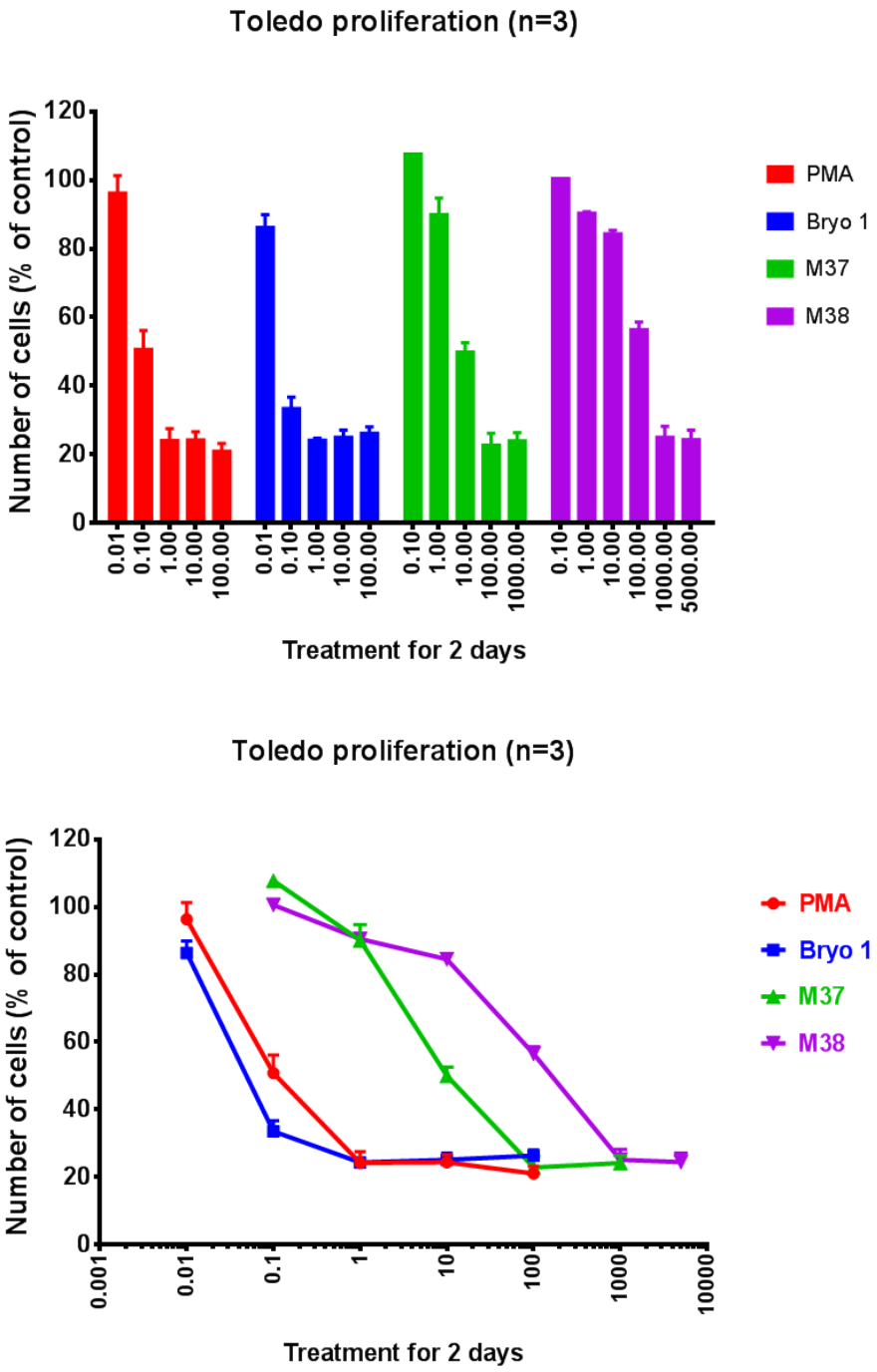


Figure 2.19. Proliferation studies in Toledo cells with Merle 37 and 38

Conclusion

Keck-Yu annulation, the flagship reaction for our analogue program proved to be a general reaction even in the presence of a free hydroxy group. Elevated temperature to -50 °C proved to be tolerated without any loss of diastereoselectivity. Our extensive structure and activity studies with Merle 34 and 37 showed that the underlying principles behind the polarity hypothesis are more complex than previously assumed. A simple correlation with clogP values with the analogue biological profile cannot be drawn without a better understanding of the role of the C13-C30 carbomethoxy enoate moiety and its role in bryostatin biology in the context of the roles of other functional groups in the northern hemisphere. This study further emphasized the argument that the northern hemisphere of bryostatin 1 is not a mere spacer domain and a subtle balance of the polar and nonpolar functional groups play an important role in the interaction of bryostatin 1 with the phospholipid and the various PKC isozymes. With the biological profile of Merle 34 and 38 in hand, we can safely rectify the diagram shown in Figure 2.5 and put them in the "intermediate" group with a tendency towards the phorbol ester like compounds (Figure 2.20). However, the greatly decreased potency of these compounds suggests that a change from sp^2 to sp^3 at C13 is not well tolerated. It would be of interest to see if this depends at all on stereochemistry at C13 in sp^3 hybridized analogues.

Experimental section

General experimental procedures, materials, and instrumentation

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals*.[19] Diisopropylamine, triethylamine, pyridine, Hünig's base,

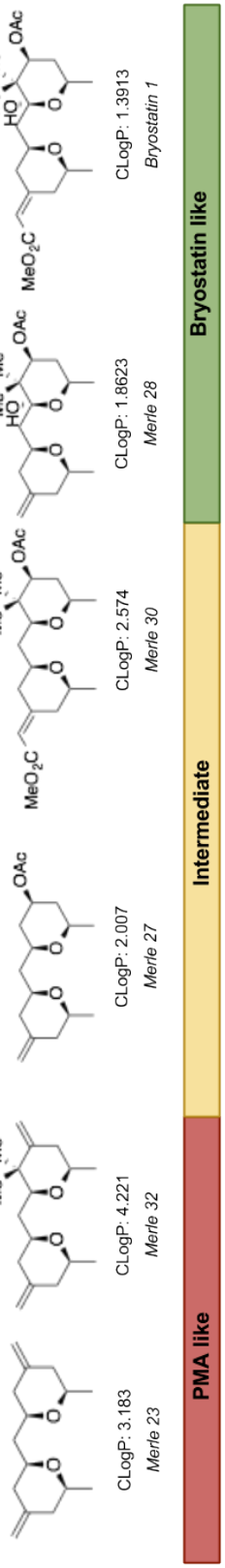
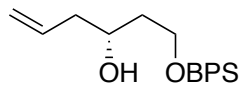


Figure 2.20. Corrected spectrum of the biological profiles of Keck analogues

EtOAc, and CH_2Cl_2 were distilled from CaH_2 under an atmosphere of dry N_2. THF, Et_2O, and toluene were distilled from Na under an atmosphere of dry N_2. Ti(O*i*Pr)$_4$ and $TiCl_4$ were distilled prior to use. A stock solution of Ti(O*i*Pr)$_4$ (1.0 M in CH_2Cl_2) was prepared and used for the BITIP catalyst preparations. The titer of *n*-butyllithium was determined by the method of Eastham and Watson.[20] All other reagents were used without further purification. Yields were calculated for material judged homogeneous by thin layer chromatography and nuclear magnetic resonance (NMR) spectroscopy. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} plates eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Glassware for reactions was oven dried at 125 ^{o}C and cooled under a dry atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septum-sealed flasks under a nitrogen atmosphere. Column flash chromatography was performed with Silicycle Grade 70 – 230 mesh, 60 – 200 μm, 60 Å silica gel, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Analtech Inc. Silica Gel GF 20 cm × 20 cm × 2000 μm plates or on Merck Kieselgel 60 F_{254} 20 cm × 20 cm × 250 μm plates. Nuclear magnetic resonance spectra were acquired on Varian VXR-500, Varian Inova-500 spectrometer 500 MHz for ^{1}H and 125 MHz for ^{13}C. Prior to use, $CDCl_3$ was filtered through a plug of Fischer Scientific 80 – 200 mesh Alumina Adsorption stored at 110 ^{o}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the signal of residual $CHCl_3$ at 7.27 ppm, or relative to the signal of residual C_6D_5H at 7.16 ppm. Chemical shifts for carbon nuclear magnetic resonance (^{13}C

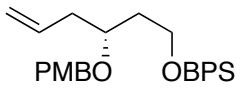
and DEPT) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the center line of the $CDCl_3$ triplet at 77.23 ppm, or relative to the center line of the C_6D_6 triplet at 128.62 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, bs, d, dd, ddd, dddd, t, td, tt, q, dq, dqd, ddq, ABq, quin, and m stand for the resonance multiplicity singlet, broad singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, doublet of quartet of doublets, doublet of doublet of quartets, AB quartet, quintet, and multiplet, respectively. IR spectra were obtained from a Perkin Elmer FT-IR Paragon 1000 PC spectrometer. Melting points were obtained using a Mel-Temp electrochemical melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin Elmer model 343 polarimeter (Na D line) using a microcell with 1 dm path length. Specific rotations ($[\alpha]^{20}_D$, Unit: $^o cm^2/g$) are based on the equation $\alpha = (100.\ \alpha)/(l.c)$ and are reported as unitless numbers where the concentration c is in g/100 mL and the path length l is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at the University of Utah on a Finnigan MAT 95 double focusing high-resolution mass spectrometer. Compounds were named using ChemBioDraw 13.0.

Experimental procedures and analytical data



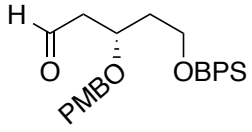
Preparation of (*S*)-1-((*tert*-butyldiphenylsilyl)oxy)hex-5-en-3-ol 2.28.[21] To a 500 mL three-neck round-bottom flask charged with a magnetic stir bar and equipped with a reflux condenser were added oven-dried 4 Å molecular sieves (34 g, 400 g/mol of aldehyde), (*R*)-BINOL (4.91g, 17.2 mmol, 0.20 equiv), CH_2Cl_2 (272 mL, 0.06 M), $Ti(OiPr)_4$ (1.00 M in CH_2Cl_2, 8.6 mL, 8.58 mmol, 0.10 equiv), and TFA (freshly prepared 1.0 M solution in CH_2Cl_2, 600 μL, 0.600 mmol, 7.0×10^{-3} equiv). The resulting red-brown solution was heated to 40 °C for 1 h. The mixture was cooled to room temperature and the reflux condenser exchanged for a rubber septum. A solution of aldehyde **2.26** (26.8 g, 85.8 mmol, 1.00 equiv) in CH_2Cl_2 (34 mL) was added via cannula, the mixture stirred for 30 min, and then cooled to -78 °C. Allyltributyl tin **2.27** (34.6 mL, 111.5 mmol, 1.30 equiv) was added, the mixture stirred for 30 min, and the flask placed in a -20 °C freezer. After 5 days, the mixture was removed from the freezer and a saturated aqueous $NaHCO_3$ solution (200 mL) was added at 0 °C. The cold bath was removed and the mixture stirred for 30 min, allowed to settle, and then filtered through a plug of Celite®. The aqueous phase was extracted with CH_2Cl_2 (2 × 250 mL) and the combined organic phase was washed with water twice (100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 5 × 24 cm silica gel column eluting with a solvent gradient of hexanes (1000 mL) and 5% acetone/ hexanes (1000 mL) collecting 25 mL fractions. The fractions containing product (25 - 44) were combined and the solvent was removed under reduced pressure to give the homoallylic alcohol **2.28** (29.5 g, 95%) as colorless oil. The ratio of the enantiomers was determined to be 98:2 (using the other enantiomer made by the same procedure, *er* = 98:2) by HPLC

analysis using a Daicel Chiralcel OD-H silica column (length: 25 cm), eluting with a mobile phase of 2.5% 2-propanol/ hexanes and a flow rate of 0.5 mL/ min, respectively, detecting with a Rainin Dynamax Refractive Index Detector Model RI-1: t_r (minor) = 8.15 min, t_r (major) = 9.21 min; R_f 0.58 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.69 – 7.62 (m, 4H), 7.45 – 7.34 (m, 6H), 5.83 (dddd, *J* = 17.2, 10.2, 7.1, 7.1 Hz, 1H), 5.12 – 5.07 (m, 1H), 5.07 – 5.05 (m, 1H), 3.98 – 3.90 (m, 1H), 3.90 – 3.78 (m, 2H), 3.15 (d, *J* = 2.6, 1H), 2.31 – 2.18 (m, 2H), 1.77 – 1.62 (m, 2H), 1.03 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 135.8, 135.8, 135.2, 133.3, 133.2, 133.0, 130.0, 128.0, 117.6, 71.0, 63.5, 42.2, 38.1, 27.0, 19.2; 125 MHz DEPT ($CDCl_3$) δ CH_3: 27.0, CH_2: 117.6, 63.5, 42.2, 38.1, CH: 135.8, 135.8, 130.0, 130.0, 128.0, 71.0.



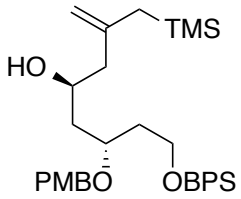
Preparation of (*S*)-*tert*-butyl((3-((4-methoxybenzyl)oxy)hex-5-en-1-yl)oxy)diphenylsilane 2.29.[21] To a stirring solution of alcohol **2.28** (5.40 g, 15.2 mmol, 1.00 equiv) and freshly prepared 4-methoxybenzyl trichloroacetimidate (10.8 g, 38.1 mmol, 2.50 equiv) in CH_2Cl_2 (31 mL, 0.50 M) in a 100 mL round-bottom flask, under an atmosphere of N_2, was added (±)-camphor-10-sulfonic acid (1.10g, 4.57 mmol, 0.30 equiv) in one portion. The reaction was allowed to proceed for 24 h at rt, after which time TLC analysis indicated essentially complete consumption of the starting material. The reaction mixture was concentrated under reduced pressure, diluted with 20% EtOAc/ hexanes (50 mL), filtered over a pad of Celite®, and concentrated under reduced pressure to give a red slurry. Purification was accomplished by flash column chromatography on a (4.5 × 30) cm silica gel column eluting with 5% EtOAc/ hexanes, collecting 25 mL fractions. The fractions containing product (15 - 27) were combined and concentrated

under reduced pressure to give PMB ether **2.29** (5.65 g, 78%) as colorless oil: R_f 0.49 (30% EtOAc/ hexanes); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 – 7.66 (m, 4H), 7.46 – 7.36 (m, 6H), 7.23 – 7.19 (m, 2H), 6.86 – 6.83 (m, 2H), 5.85 (dddd, *J* = 17.3, 10.3, 7.0, 7.0 Hz, 1H), 5.12 – 5.05 (m, 2H), 4.45 (ABq, *J* = 11.1 Hz, Δν = 44.9 Hz, 2H), 3.86 – 3.83 (m, 1H), 3.80 (s, 3H), 3.78 – 3.69 (m, 2H), 2.33 (t, *J* = 7.0 Hz, 2H), 1.87 – 1.77 (m, 2H), 1.10 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 159.3, 135.8, 135.1, 134.2, 134.1, 131.2, 129.8, 129.5, 127.8, 127.8, 117.1, 113.9, 75.3, 71.0, 60.7, 55.5, 38.7, 37.2, 27.1, 19.4; 125 MHz DEPT ($CDCl_3$) δ CH_3: 55.5, 27.1, CH_2: 117.1, 71.0, 60.7, 38.7, 37.2, CH: 135.8, 135.1, 129.8, 129.5, 127.8, 127.8, 113.9, 75.3.



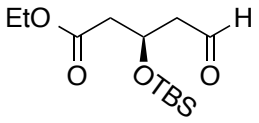
Preparation of (*S*)-5-(*tert*-butyldiphenylsilyloxy)-3-(4-methoxybenzyloxy) pentanal 2.19.[22] To a stirring solution of alkene **2.29** (447 mg, 0.941 mmol, 1.0 equiv) in a mixture of 20% MeOH/CH_2Cl_2 (100 mL) in a 50 mL round-bottom flask was added sodium bicarbonate (790 mg) in one portion. The reaction mixture was cooled to -78 ºC, and then a steady stream of ozone was bubbled into the solution for 1 min, during which time the color changed to light grey. The mixture was then purged with a steady stream of oxygen. Dimethyl sulfide (2.8 mL, 37.6 mmol, 40.0 equiv) was added in one portion to the solution, which was then allowed to warm to rt over a period of 1 h. Solid $NaHCO_3$ was removed by filtration and the solution was concentrated under reduced pressure to give a yellow oil. Purification was accomplished by flash chromatography on a 2.5 × 10 cm silica gel column, eluting with 20% EtOAc/hexanes (600 mL), collecting 9 mL fractions. The fractions containing product (15-39) were combined and concentrated

under reduced pressure to give the product **2.46** (404 mg, 90%) as colorless oil: 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.75 (t, *J* = 2.0 Hz, 1H), 7.69-7.62 (m, 4H), 7.48-7.34 (m, 6H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz,, 2H), 4.45 (s, 2H), 4.19 (dddd, *J* = 6.6, 6.6, 6.4, 5.7 Hz, 1H), 3.91-3.86 (m, 1H), 3.84 (s, 3H), 3.82-3.76 (m, 1H), 2.69 (ddd, *J* = 16.1, 7.1, 2.4 Hz, 1H), 2.63 (ddd, *J* = 16.4, 6.7, 1.7 Hz, 1H), 2.00-1.91 (m, 1H), 1.87-1.80 (m, 1H), 1.08 (s, 9H). 125 MHZ ^{13}C NMR ($CDCl_3$) δ 201.6, 159.5, 135.7, 133.8, 133.7, 130.4, 129.9, 129.8, 129.6, 127.9, 113.9, 71.4, 71.3, 60.3, 55.4, 48.7, 37.3, 27.1, 19.3.



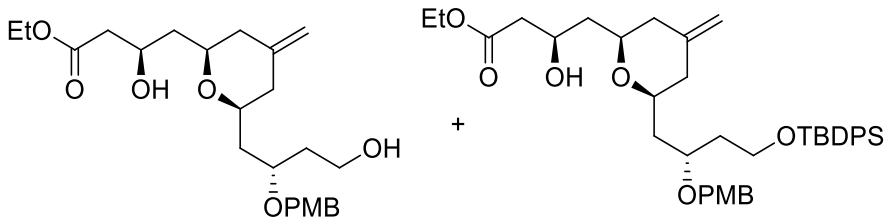
Preparation of (4*S*, 6*S*)-8-(tert-butyldiphenylsilyloxy)-6-(4-methoxybenzyloxy) -2-((trimethylsilyl)methyl)oct-1-en-4-ol 2.17.[22] To a solution of aldehyde **2.19** (5.0 g, 10.5 mmol, 1.0 equiv) in CH_2Cl_2 (105 mL, 0.1 M) in a 250 mL round-bottom flask was added $MgBr_2 \cdot OEt_2$ (5.4 g, 21.0 mmol, 2.0 equiv) in one portion at rt. After 5 min at rt, the reaction mixture was cooled to -78 ºC and stirred for 30 min. Stannane **2.18** (8.76 g, 21.0 mmol. 2.0 equiv) was added dropwise via syringe. After 5 h at -78 ºC, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (25 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 3 × 25 cm silica gel column, eluting with 8% acetone/hexanes (800 mL), collecting 9 mL fractions. The fractions containing product (30-59) were combined and concentrated under reduced pressure to give the product **2.17**

(4.7 g, 74%) as colorless oil: 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.46-7.34 (m, 6H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 4.65 (dd, *J* = 1.1, 0.8 Hz, 1H), 4.66 (s, 1H), 4.48 (s, 2H), 4.04-3.93 (m, 2H), 3.85-3.79 (m, 1H), 3.81 (s, 3H), 3.76 (dt, *J* = 10.0, 5.8 Hz, 1H), 2.67 (d, *J* = 2.2 Hz, 1H), 2.12 (dd, *J* = 14.1, 8.2 Hz, 1H), 2.06 (dd, *J* = 13.8, 5.0 Hz, 1H), 1.94 (dq, *J* = 12.8, 6.0 Hz, 1H), 1.79 (dq, *J* = 13.8, 6.7 Hz, 1H), 1.72-1.60 (m, 2H), 1.55 (ABq, *J* = 13.4 Hz, Δν = 18.1 Hz, 2H), 1.08 (s, 9H), 0.04 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 159.6, 144.9, 135.8, 134.0, 139.9, 130.8, 129.8, 129.8, 127.9, 127.8, 127.7, 114.0, 110.2, 74.0, 71.6, 66.5, 60.7, 55.6, 47.0, 40.8, 37.3, 27.1, 26.9, 19.4, -1.1.



Preparation of ethyl (*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-oxopentanoate 2.15.[22] To a stirring solution of the corresponding alcohol of **2.34** (167 mg, assumed to be 0.604 mmol, 1 equiv, supplied by Dr. Wei Li) in CH_2Cl_2 (6 mL, 0.1 M) in a 25 mL round-bottom flask was added diisopropylethylamine (734 µL, 4.22 mmol, 7.0 equiv). The reaction mixture was cooled to -10 ºC, and then dimethyl sulfoxide (430 µL, 6.04 mmol, 10.0 equiv) was added via syringe. After 5 min at -10 ºC, SO_3·Py (384 mg, 2.42 mmol, 4.0 equiv) was added in one portion. After 1 h at -10 ºC, the reaction mixture was poured into a 250 mL Erlenmeyer flask containing 25 mL of saturated aqueous $NaHCO_3$ solution. The reaction mixture was stirred at rt for 1 h, then the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were combined and washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 2 ×

21 cm silica gel column, eluting with 5% EtOAc/hexanes (600 mL), collecting 9 mL fractions. The fractions containing product (9-27) were combined and concentrated under reduced pressure to provide the aldehyde **2.15** (135 mg, 96% over 2 steps) as colorless oil: R_f = 0.52 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.81 (t, *J* = 2.0 Hz, 1H), 4.63 (dddd, *J* = 6.2, 6.2, 5.9, 5.9 Hz, 1H), 4.13 (m, 2H), 2.67 (ddd, *J* = 16.6, 5.3, 1.7 Hz, 1H), 2.61 (ddd, *J* = 16.6, 6.4, 2.4 Hz, 1H), 2.55 (dd, *J* = 15.1, 6.4 Hz, 1H), 2.55-2.50 (dd, *J* = 15.1, 6.4 Hz, 1H), 1.25 (t, *J* = 6.8 Hz, 3H), 0.84 (s, 9H), 0.07 (s, 3H), 0.07 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 201.1, 170.8, 65.2, 60.9, 51.1, 42.8, 25.8, 18.1, 14.4, -4.6, -4.6; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 25.8, 14.3, -4.6, -4.6; CH_2 δ 60.8, 51.1, 42.8; CH δ 201.1, 65.2;



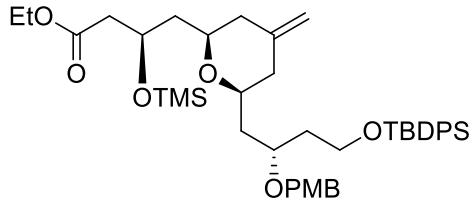
Preparation of (*R*)-ethyl-4-((2*R*,6*S*)-6-((*S*)-4-(hydroxy)-2-(4-methoxy benzyl oxy) butyl)-4-methylenetetra hydro-2H-pyran-2-yl)-3-hydroxy butanoate and (*R*)-ethyl 4-((2*R*,6*S*)-6-((*S*)-4-(*tert*-butyldiphenyl silyloxy)-2-(4-methoxybenzyloxy)butyl)-4-methylene tetrahydro-2H-pyran-2-yl)-3-hydroxy butanoate 2.36 and 2.35.[22] To a stirring solution of silyl ether **2.14** (938 mg, 1.18 mmol, 1.0 equiv) in a mixture of 3:2 benzene/MeOH (13.0 mL, 0.1 M) in a 50 mL round-bottom flask was added *p*-toluenesulfonic acid (451 mg, 2.37 mmol, 2.0 equiv) in one portion. After 3 h at rt, the reaction mixture was quenched by the addition of triethylamine (2 mL), and then with saturated aqueous $NaHCO_3$ solution. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layer was washed with

brine (25 mL) and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 3×20 cm silica gel column, eluting with 20% EtOAc/hexanes (900 mL) and 45% EtOAc/hexanes (500 mL), collecting 20 mL fractions. The fractions containing product (23-43) were combined and concentrated under reduced pressure to give the mono-deprotected product **2.35** (545 mg, 68%) as colorless oil: R_f = 0.48 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.62 (m, 4H), 7.47-7.35 (m, 6H), 7.20 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 4.73 (d, *J* = 1.7 Hz, 1H), 4.40 (ABq, *J* = 11.1 Hz, Δν = 40.4 Hz, 2H), 4.30-4.24 (m, 1H), 4.18-4.12 (m, 2H), 3.83-3.75 (m, 7H), 3.57-3.51 (m, 2H), 2.55 (dd, *J* = 15.6, 7.7 Hz, 1H), 2.45 (dd, *J* =15.6, 5.4 Hz, 1H), 2.25 (d, *J* = 13.1 Hz, 1H), 2.17 (d, *J* = 13.1 Hz, 1H), 2.00 (t, *J* = 12.1 Hz, 1H), 1.94 (t, *J* = 11.4 Hz, 1H), 1.86-1.64 (m, 6H), 1.25 (t, *J* = 7.4 Hz, 3H), 1.08 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.1, 159.3, 143.9, 135.8, 134.0, 131.0, 129.8, 129.7, 127.8, 114.0, 109.2, 78.3, 75.4, 72.7, 71.5, 67.8, 60.7, 60.5, 55.4, 42.5, 42.1, 41.9, 41.0, 37.5, 27.1, 19.3, 14.4; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.4, 27.1, 14.4; CH_2 δ 109.2, 71.6, 60.7, 60.5, 42.5, 42.1, 41.9, 41.0, 41.0, 37.5; CH δ 135.8, 129.8, 129.7, 127.9, 114.0, 78.4, 75.5, 72.8, 67.8.

The fractions containing product (55-75) were combined and concentrated under reduced pressure to provide the di-deprotected product **2.36** (150 mg, 29%) as colorless oil: R_f = 0.38 (EtOAc); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.26 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.75 (s, 2H), 4.48 (ABq, *J* = 11.2, Δν = 36.4 Hz, 2H), 4.27-4.21 (m, 1H), 4.16 (q, *J* = 7.3 Hz, 2H), 3.83-3.75 (m, 2H), 3.79 (s, 3H), 3.74-3.67 (m, 1H), 3.55-3.49 (m, 2H), 2.46 (dd, *J* = 15.6, 7.8 Hz, 1H), 2.44 (dd, *J* = 15.6, 5.4 Hz, 1H), 2.27 (bs, 1H), 2.25-2.16 (m, 2H), 2.00 (d, *J* = 12.2 Hz, 1H),1.95 (q, *J* = 11.7 Hz, 1H), 1.92-1.85 (m,

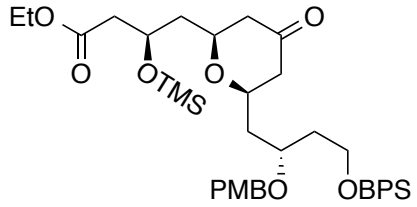
1H), 1.79-1.64 (m, 5H), 1.24 (t, J = 7.3 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.1, 159.6, 143.7, 130.7, 129.9, 114.2, 109.4, 78.4, 75.6, 74.5, 71.5, 67.8, 60.8, 60.1, 55.6, 42.5, 42.1, 41.4, 41.1, 41.0, 36.6, 14.3; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.5 14.4; CH_2 δ 109.5, 71.6, 60.8, 60.1, 42.5, 42.1, 41.4, 41.1, 41.0, 36.6; CH δ 129.9, 114.1, 78.5, 75.8, 74.5, 67.8.

To a stirring solution of diol **2.36** (110 mg, 0.253 mmol, 1.0 equiv) in CH_2Cl_2 (2.5 mL, 0.1 M) in a 15 mL round-bottom flask were added DMAP (8 mg, 0.063 mmol, 0.25 equiv), BPSCl (98.6 μL, 0.380 mmol, 1.5 equiv), and Et_3N (53 μL, 0.380 mmol, 1.5 equiv) via syringe. After 12 h at rt, the reaction mixture was quenched by the addition of water (5.0 mL). The phases were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 1 × 10 cm silica gel column, eluting with 20% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (10-14) were combined and concentrated under reduced pressure to provide the alcohol **2.35** (162 mg, 95%) as colorless oil.



Preparation of (*R*)-ethyl 4-((2*R*,6*S*)-6-((*S*)-4-(*tert*-butyldiphenyl silyloxy)-2-(4-methoxybenzyloxy)butyl)-4-methylenetetrahydro-2H-pyran-2-yl)-3-(trimethyl silylo xy) butanoate 2.37.[22] To a stirring solution of alcohol **2.35** (297 mg, 0.440 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL, 0.1 M) in a 15 mL round-bottom flask were added TMSCl

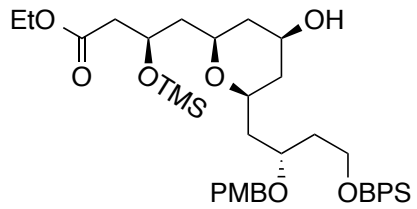
(167.5 μL, 1.32 mmol, 3.0 equiv) and triethylamine (367 μL, 2.64 mmol, 6.0 equiv) dropwise via syringe. After 12 h at rt, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (2 mL). The phases were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 3 × 17 cm silica gel column, eluting with 10% EtOAc/hexanes (400 mL), collecting 9 mL fractions. The fractions containing product (15-21) were combined and concentrated under reduced pressure to provide the silyl ether **2.37** (322 mg, 98%) as a colorless oil: R_f = 0.60 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.76-7.72 (m, 4H), 7.49-7.41 (m, 6H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 4.76 (d, *J* = 1.0 Hz, 1H), 4.74 (s, 1H), 4.47 (ABq, *J* = 11.7 Hz, Δν = 28.4 Hz, 2H), 4.43-4.39 (m, 1H), 4.18-4.06 (m, 2H), 3.93 (m, 1H), 3.86-3.78 (m, 5H), 3.60-3.52 (m, 1H), 3.48-3.40 (m, 1H), 2.53 (d, *J* = 1.0 Hz, 1H), 2.53 (s, 1H), 2.30 (d, *J* = 12.7 Hz, 1H), 2.22 (d, *J* = 12.8 Hz, 1H), 2.01 (d, *J* = 13.0 Hz, 1H), 1.95 (d, *J* = 12.4 Hz, 1H), 1.94-1.84 (m, 3H), 1.72-1.66 (m, 3H), 1.22 (t, *J* = 7.3 Hz, 3H), 1.10 (s, 9H), 0.16 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.7, 159.2, 144.7, 135.8, 134.1, 134.0, 131.2, 129.7, 129.5, 127.8, 113.9, 108.7, 75.1, 72.8, 71.7, 66.8, 60.7, 60.4, 55.4, 44.2, 43.0, 42.4, 41.3, 41.1, 37.8, 27.1, 19.3, 14.4, 0.5.



Preparation of (*R*)-ethyl 4-((2*S*,6*R*)-6-((*S*)-4-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxy benzyloxy)butyl)-4-oxotetrahydro-2H-pyran-2-yl)-3-(trimethylsilyloxy) but

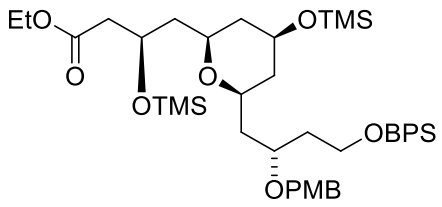
anoate 2.38.[22] To a stirring solution of alkene **2.37** (265 mg, 0.356 mmol, 1.0 equiv) in CH_2Cl_2 (35 mL, 0.01 M) in a 50 ml round-bottom flask was added $NaHCO_3$ (200 mg). The mixture was cooled to -78 ºC, and then a steady stream of ozone was bubbled through the solution for 1 min, during which time the solution developed a light grey color. The solution was then purged with a steady stream of oxygen until the grey color disappeared. PPh_3 (280 mg, 1.06 mmol, 3.0 equiv) was added in one portion, and the reaction mixture was allowed to warm to rt and stirred overnight. The solid $NaHCO_3$ was removed by filtration and the filtrate was concentrated under reduced pressure to give yellow oil. Purification was accomplished by flash chromatography on a 3 × 21 cm silica gel column, eluting with 15% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (15-19) were combined and concentrated under reduced pressure to give the product **2.38** (242 mg, 91% yield) as a colorless oil: R_f = 0.37 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) 7.75-7.65 (m, 4H), 7.49-7.35 (m, 6H), 7.17 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 4.47 (d, *J* = 11.1 Hz, 1H), 4.38-4.32 (m, 1H), 4.36 (d, *J* = 10.7 Hz, 1H), 4.11 (dddd, *J* = 10.6, 7.1, 7.1, 7.1 Hz, 1H), 4.05 (dddd, *J* = 10.6, 7.1, 7.1, 7.1 Hz, 1H), 3.93 (dddd, *J* = 9.1, 5.6, 5.6, 3.0 Hz, 1H), 3.83 (dddd, *J* = 9.1, 9.1, 2.7, 2.7 Hz, 1H), 3.84-3.78 (m, 5H), 3.76-3.70 (m, 2H), 2.53 (dd, *J* = 14.8, 7.7 Hz, 1H), 2.48 (dd, *J* = 14.8, 7.7 Hz, 1H), 2.40 (ddd, *J* = 14.4, 2.0, 2.0 Hz, 1H), 2.33 (ddd, *J* = 14.4, 2.0, 2.0 Hz, 1H), 2.25 (dd, *J* = 14.1, 11.8 Hz, 1H), 2.20 (dd, *J* = 14.1, 11.8 Hz, 1H), 1.96-1.86 (m, 2H), 1.84-1.73 (m, 2H), 1.72-1.62 (m, 2H), 1.20 (t, *J* = 7.4 Hz, 3H), 1.06 (s, 9H), 0.10 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 207.0, 171.5, 159.3, 135.8, 135.8, 134.0, 134.0, 131.0, 129.8, 129.4, 127.9, 127.9, 113.9, 73.8, 73.6, 72.7, 71.6, 66.4, 60.6, 60.5, 55.5, 48.3, 48.1, 43.9, 42.8, 42.6, 37.4, 27.1, 19.4, 14.4, 0.4; 125 MHz DEPT

^{13}C NMR ($CDCl_3$) CH_3 δ 55.5, 27.1, 14.4, 0.4; CH_2 δ 71.6, 60.6, 60.6, 48.3, 48.1, 44.0, 42.8, 42.6, 37.4; CH δ 135.8, 129.8, 129.5, 129.5, 127.9, 73.8, 73.6, 72.7, 66.4.



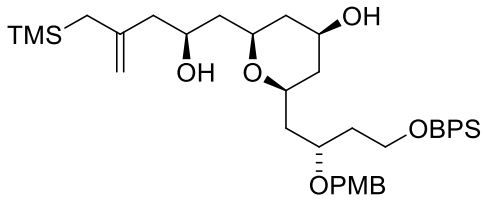
Preparation of (*R*)-ethyl 4-((2*R*,4*S*,6*S*)-6-((*S*)-4-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxy benzyloxy)butyl)-4-hydroxytetrahydro-2H-pyran-2-yl)-3-(trimethylsilyl oxy) butanoate 2.40.[22] To a solution of ketone **2.38** (32.0 mg, 0.043 mmol, 1.0 equiv) in MeOH (3.0 mL, 0.015M) in a 15 mL round-bottom flask at -15 ºC was added $NaBH_4$ (3.2 mg, 0.086 mmol, 2.0 equiv) in one portion. After 30 min at -15 ºC, the mixture was quenched by the addition of acetone (0.1 mL), and then concentrated under reduced pressure. Purification was accomplished by flash chromatography column on a 1 × 10 cm silica gel column, eluting with 40% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (15-20) were combined and concentrated under reduced pressure to give the alcohol product **2.40** (30.0 mg, 94%) as a colorless oil: R_f = 0.15 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.74-7.63 (m, 4H), 7.46-7.34 (m, 6H), 7.18 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.45 (d, *J* = 10.8 Hz, 1H), 4.38 (d, *J* = 11.1 Hz, 1H), 4.40-4.32 (m, 1H), 4.10 (dddd, *J* = 10.6, 7.1, 7.1, 7.1 Hz, 1H), 4.05 (dddd, *J* = 10.6, 7.1, 7.1, 7.1 Hz, 1H), 3.91-3.86 (m, 1H), 3.82-3.74 (m, 7H), 3.56-3.50 (m, 1H), 3.46-3.39 (m, 1H), 2.50 (d, *J* = 5.5 Hz, 1H), 2.49 (d, *J* = 3.0 Hz, 1H), 1.97 (ddd, *J* = 12.4, 4.4, 2.4 Hz, 1H), 1.90-1.76 (m, 4H), 1.68-1.45 (m, 5H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.06 (s, 9H), 0.12 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.8, 159.3, 135.8, 135.7, 134.1, 134.1, 131.2, 131.2, 129.8, 129.6, 127.9, 127.9, 113.9, 72.9, 72.1,

72.1, 71.7, 68.3, 66.8, 60.7, 60.5, 55.6, 43.9, 42.9, 42.3, 41.8, 41.5, 37.8, 27.1, 19.4, 14.4, 0.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.6, 27.1, 14.4, 0.5; CH_2 δ 71.7, 60.7, 60.5, 43.9, 42.9, 42.3, 41.8, 41.5, 37.8; CH δ 135.8, 135.8, 129.8, 129.5, 127.9, 127.9, 114.0, 72.9, 72.1, 68.4, 66.8.



Preparation of (*R*)-ethyl 4-((2*R*,4*S*,6*R*)-6-((*S*)-4-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxy benzyloxy)butyl)-4-(trimethylsilyloxy) tetrahydro-2H-pyran-2-yl)-3-(trimethylsilyloxy) butanoate 2.13.[22] To a solution of alcohol **2.40** (36.4 mg, 0.048 mmol, 1.0 equiv) in CH_2Cl_2 (500 μL, 0.1 M) in a 10 mL round-bottom flask were added TMSCl (31 μL, 0.243 mmol, 5.0 equiv) and Et_3N (67.5 μL, 0.480 mmol, 10.0 equiv) dropwise via syringe. After 12 h at rt, the reaction mixture was quenched by the addition of water (5 mL). The phases were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 1 × 10 cm silica gel column, eluting with 10% EtOAc/hexanes (200 mL), collecting 9 mL fractions. The fractions containing product (8-12) were combined and concentrated under reduced pressure to give the product **2.13** (39.2 mg, 98% yield) as a colorless oil: R_f = 0.62 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70 (ddd, *J* = 4.4, 1.4, 1.4 Hz, 2H), 7.67 (dd, *J* = 4.0, 1.7 Hz, 2H), 7.45-7.35 (m, 6H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 4.41 (ABq, *J* = 11.1 Hz, Δν = 41.0 Hz, 1H), 4.37-4.30 (m, 1H), 4.10 (dddd, *J* = 10.7, 7.1, 7.1, 7.1 Hz, 1H), 4.05 (dddd, *J* = 10.7, 7.1, 7.1,

7.1 Hz, 1H), 3.90-3.84 (m, 1H), 3.80 (s, 3H), 3.80-3.71 (m, 3H), 3.56-3.50 (m, 1H), 3.45-3.38 (m, 1H), 2.50 (d, *J* = 4.7 Hz, 1H), 2.48 (d, *J* = 2.7 Hz, 1H), 1.86-1.72 (m, 5H), 1.66-1.54 (m, 3H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.05 (s, 9H), 0.13 (s, 9H), 0.11 (s, 9H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 171.8, 159.3, 135.8, 135.8, 134.1, 134.1, 129.8, 129.6, 127.9, 114.0, 72.9, 72.2, 72.1, 71.8, 68.8, 66.8, 60.7, 60.5, 55.5, 44.0, 43.1, 42.3, 42.3, 41.9, 37.7, 27.1, 19.4, 14.4, 0.5, 0.5; 125 MHz DEPT ^{13}C NMR ($CDCl_3$) CH_3 δ 55.5, 27.1, 14.4, 0.5, 0.5; CH_2 δ 71.8, 60.7, 60.5, 44.0, 43.1, 42.3, 42.3, 41.9, 37.7; CH δ 135.8, 129.8, 129.6, 127.9, 114.0, 72.9, 72.2, 72.1, 68.8, 66.8.



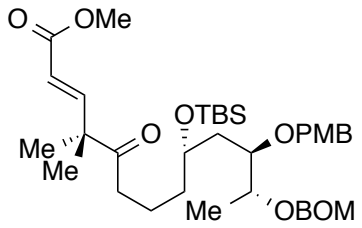
Preparation of (2*S*,4*R*,6*S*)-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxy benzyloxy) butyl)-6-((*S*)-2-hydroxy-4-((trimethylsilyl) methyl)pent-4-enyl)tetrahydro-2H-pyran-4-ol 2.12.[22] A 15 mL round-bottom flask was charged with $CeCl_3 \bullet 7H_2O$ (453.0 mg, 1.21 mmol, 10.0 equiv) and a stir bar. The reaction flask was heated to 170 ºC under 0.1 mm Hg vacuum. After 16 h at 170 ºC, the dried $CeCl_3$ was cooled to rt, and the flask was purged with N_2. THF (1.2 mL) was added via syringe, and the mixture was stirred at rt for 2 h.

Preparation of the Grignard reagent: Another 15 mL three-necked round-bottom flask equipped with a condenser and a magnetic stir bar was charged with shiny magnesium turnings (122.0 mg, 5 mmol, 1.0 equiv), and a crystal of iodine. The flask was heated with a heat gun for 5 min while stirring. THF (5.0 mL, 1 M) was added via syringe, and the reaction mixture was heated with a heat gun at reflux. $TMSCH_2Cl$ (768

μL, 5.5 mmol, 1.1 equiv) was then added dropwise via syringe. The mixture was then stirred at rt for 1.5 h to give an assumed 1.0 M of 5 mL solution of $TMSCH_2MgCl$.

The $CeCl_3$/THF mixture was cooled to -78 ºC, then a solution of $TMSCH_2MgCl$ (1.1 mL, 1.0 M in THF, 1.2 mmol, 10.0 equiv) was added dropwise via syringe. After 1 h at -78 ºC, a solution of ester **2.13** (100 mg, 0.121 mmol, 1.0 equiv) in THF (243 μL, 0.5 M) was then added via cannula. An additional THF (500 μL) rinse was used to transfer the remaining ester residue into the reaction mixture. The solution was allowed to warm to rt and stirred overnight. The mixture was again cooled to -78 ºC, and then chilled 1N HCl solution (4.0 mL) was added dropwise via syringe. The reaction mixture was then warmed to rt and the phases were separated. The aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, washed with saturated aqueous $NaHCO_3$ solution (10 mL), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 1 × 10 cm silica gel column, eluting with 50% EtOAc/hexanes (400 mL), collecting 9 mL fractions. The fractions containing product (9-25) were combined and concentrated under reduced pressure to give the product **2.12** (61.2 mg, 70%) as a colorless oil: R_f = 0.31 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.74-7.63 (m, 4H), 7.45-7.37 (m, 6H), 7.20 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 2H), 4.67 (s, 1H), 4.64 (s, 1H), 4.48-4.43 (ABq, *J* = 11.1 Hz, Δν = 42.5, 1H), 4.00-3.94 (m, 1H), 3.81-3.74 (m, 6H), 3.60-3.50 (m, 2H), 3.44 (s, 1H), 2.22 (dd, *J* = 13.8, 7.1 Hz, 1H), 2.07 (dd, *J* = 13.8, 6.0 Hz, 1H), 1.96 (ddd, *J* = 12.1, 2.4, 2.0 Hz, 1H), 1.87 (ddd, *J* = 12.4, 2.4, 2.4 Hz, 1H), 1.84-1.78 (m, 3H), 1.72-1.60 (m, 5H), 1.57 (s, 2H), 1.07 (s, 9H), 0.05 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 159.3, 144.6, 135.8, 134.0, 134.0, 131.1, 129.8, 129.7, 127.8, 127.8 114.0,

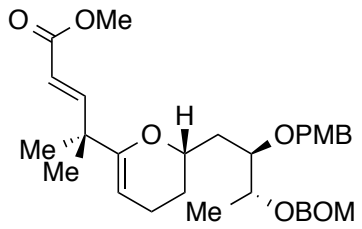
110.1, 76.3, 72.8, 72.5, 71.6, 69.5, 67.8, 60.5, 55.4, 46.6, 42.4, 41.8, 41.6, 41.5, 37.5, 27.1, 27.1, 19.3, -1.2.



Preparation of (9*S*,11*R*,12*R*,*E*)-methyl-12-(benzyloxymethoxy)-9-(*tert*-butyl dimethylsilyloxy)-11-(4-methoxybenzyloxy)-4,4-dimethyl-5-oxotridec-2-enoate 2.42.[22] To a stirring solution of alkene **2.23** (1.48 g, 2.41 mmol, 1.0 equiv) in 5% MeOH/EtOAc (50 mL, 0.05 M) in a 100 mL round-bottom flask at -78 °C was added $NaHCO_3$ (2 g, 24.1 mmol, 10.0 equiv). A steady stream of O_3 was bubbled through the reaction mixture until a light blue color developed. The excess O_3 was removed by bubbling O_2 through the mixture for 15 min until the light blue color faded. PPh_3 (945 mg, 3.60 mmol, 1.5 equiv) was added in one portion, and the reaction mixture was slowly warmed to rt, and stirred for 12 h. The solids were removed via filtration, and the filtrate was concentrated under reduced pressure. The resulting yellow oil was taken up in 10% Et_2O/ pentane (200 mL) in a 500 mL round-bottom flask, and placed in a -20 °C freezer for 6 h. The triphenylphosphine oxide precipitate was removed via filtration, and rinsed with 100 mL of ice cold 1% Et_2O/pentane. The solvent was removed under reduced pressure to yield the crude aldehyde as light yellow oil, which was taken on to the next step without further purification.

To a stirring solution of methyl diethylphosphonoacetate **2.41** (960 μL, 5.30 mmol, 2.2 equiv) in THF (12 mL, 0.2 M) in a 50 mL round-bottom flask at 0 °C was added NaH (127 mg, 5.30 mmol, 2.2 equiv) portion wise over 10 min. The reaction

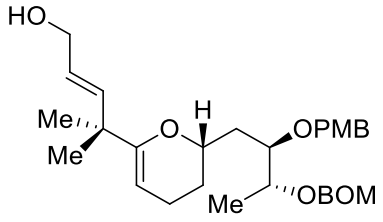
mixture was stirred at 0 °C for 30 min, and then a solution of the aforementioned crude aldehyde in THF (5 mL) was added to the mixture slowly via cannula. The transfer was completed with two 2.5 mL rinses using THF. The reaction mixture was stirred at 0 °C for an additional 2 h, then quenched by the addition of saturated aqueous NH_4Cl solution (15 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried with Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 3 × 21 cm silica gel column, eluting with 10% EtOAc/hexanes (800 mL), collecting 9 mL fractions. The fractions containing product (17-43) were combined and concentrated under reduced pressure to give the product **2.42** (1.50 g, 92% over 2 steps) as a colorless oil: R_f = 0.50 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.34 (m, 4H), 7.31-7.28 (m, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 15.4 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.90 (d, *J* = 15.4 Hz, 1H), 4.81 (ddd, *J* = 7.3, 6.8, 6.4 Hz, 2H), 4.64 (q, *J* = 11.7 Hz, 2H), 4.58 (d, *J* = 11.2 Hz, 1H), 4.45 (d, *J* = 10.7 Hz, 1H), 4.02 (dd, *J* = 16.4, 14.9 Hz, 1H), 3.89 (dddd, *J* = 8.8, 8.8, 5.4 Hz, 1H), 3.80 (s, 3H), 3.76 (s, 3H), 3.64 (ddd, *J* = 9.4, 4.4, 2.4 Hz, 1H), 2.42 (ddd, *J* = 7.3, 7.3, 2.0 Hz, 2H), 1.69 (ddd, *J* = 14.2, 8.8, 2.4 Hz, 1H), 1.62-1.54 (m, 3H), 1.47-1.40 (m, 2H), 1.27 (s, 6H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) 210.8, 166.9, 159.2, 152.0, 138.1, 131.1, 129.3, 128.6, 128.0, 127.8, 120.5, 113.9, 93.4, 78.2, 73.0, 72.1, 69.6, 69.5, 55.4, 51.9, 50.7, 38.5, 37.8, 37.4, 26.1, 23.7, 19.2, 18.3, 15.2, -3.5, -4.2.



Preparation of (*E*)-methyl 4-((*S*)-2-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxybenzyloxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-4-methylpent-2-enoate 2.21.[22] To a stirring solution of silyl ether **2.42** (1.42 g, 2.12 mmol, 1.0 equiv) in 20:1 CH_3CN/ H_2O (42 mL, 0.05 M) in a 100 mL plastic bottle at 0 °C were added pyridine (7 mL) and a 48% aqueous HF solution (500 μL). The solution was stirred at 0 °C for 30 min, and then warmed to rt. After 30 min of stirring at rt, additional 500 μL of aqueous HF solution (48%) was added every hour until TLC analysis indicated complete consumption of the starting material. The reaction mixture was quenched by slowly pipetting the solution into a mixture of saturated aqueous $NaHCO_3$ solution (50 mL) and EtOAc (50 mL). Then solid $NaHCO_3$ was added until effervescence was complete. The phases were separated and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with water (100 mL), saturated aqueous $CuSO_4$ solution (2 × 20 mL), and brine (2 × 20 mL). The solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude intermediate alcohol as light yellow oil. This intermediate was carried on to the next step without further purification.

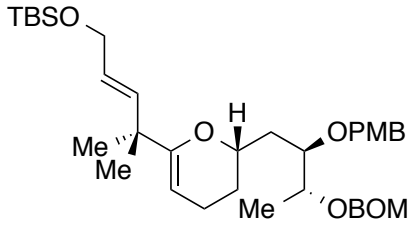
To a stirring solution of the aforementioned crude alcohol (assumed to be 2.12 mmol) in benzene (42 mL, 0.05 M) in a 50 mL round-bottom flask equipped with a condenser and a Dean-Stark apparatus was added CSA (24.5 mg, 0.106 mmol, 0.05 equiv). The solution was heated at reflux for 1.5 h, and then cooled to rt. The reaction mixture was quenched by the addition of pyridine (0.1 mL), and the solvent was removed under reduced pressure. Purification was accomplished using flash chromatography on a 3.0 × 21 cm silica gel column, eluting with 10% EtOAc/hexanes (1000 mL), collecting 9 mL fractions. The fractions containing product (24-65) were combined and concentrated

under reduced pressure to give the product dihydropyran **2.21** (823.2 mg, 73% over 2 steps) as a colorless oil: R_f = 0.42 (20% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.35 (m, 4H), 7.33-7.29 (m, 1H), 7.27 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 15.6 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.84 (d, *J* = 15.4 Hz, 1H), 4.83 (ABq, *J* = 8.8 Hz, Δν = 11.7 Hz, 2H), 4.65 (ABq, *J* = 11.7 Hz, Δν = 14.2 Hz, 2H), 4.62 (d, *J* = 10.7 Hz, 1H), 4.63-4.63 (m, 1H), 4.50 (d, *J* = 10.3 Hz, 1H), 4.01-3.97 (m, 1H), 3.97 (dd, *J* = 6.5, 5.4 Hz, 1H), 3.82 (dd, *J* = 5.4, 2.0 Hz, 1H), 3.80 (s, 3H), 3.64 (s, 3H), 2.14-2.06 (m, 1H), 2.04-1.96 (m, 1H), 1.84-1.74 (m, 2H), 1.62 (ddd, *J* = 14.2,10.7, 2.4 Hz, 1H), 1.52 (dddd, *J* = 13.2, 9.8, 9.8, 5.9 Hz, 1H), 1.24 (s, 3H), 1.22 (s, 3H), 1.21 (d, *J* = 6.4 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) 167.6, 159.3, 157.2, 156.4, 138.2, 131.1, 129.6, 128.6, 128.0, 127.8, 117.9, 114.0, 94.5, 93.6, 77.7, 73.9, 73.6, 72.0, 69.5, 55.5, 51.5, 41.4, 36.2, 28.2, 25.3, 25.2, 20.5, 15.6.



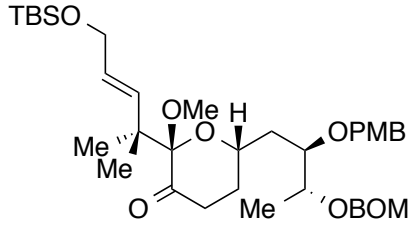
Preparation of (*E*)-4-((*S*)-2-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy)butyl)-3,4-dihydro-2H-pyran-6-yl)-4-methylpent-2-en-1-ol 2.43.[22] To a stirring solution of ester **2.21** (794 mg, 1.47 mmol, 1.0 equiv) in CH_2Cl_2 (15 mL, 0.1 M) at -78 °C was added a solution of DIBAL-H (2.1 mL, 1.5 M in toluene, 3.10 mmol, 2.1 equiv) slowly via syringe. The solution was stirred at -78 °C for 2 h, then warmed to 0 °C and stirred for 0.5 h. The reaction was quenched by the addition of EtOAc (0.5 mL) and the mixture was stirred at 0 °C for 10 min. A saturated Rochelle salt solution (10 mL) was added. Then the aqueous phase was separated and extracted with CH_2Cl_2 (3 × 25

mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 3 × 21 cm silica gel column, eluting with 20% EtOAc/hexanes (500mL), collecting 9 mL fractions. The fractions containing product (19-42) were combined and concentrated under reduced pressure to give the product alcohol **2.43** (587.1 mg, 78%) as a clear colorless oil: R_f = 0.30 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.34-7.30 (m, 4H), 7.28-7.25 (m, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 5.74 (d, *J* = 16.1 Hz, 1H), 5.58 (ddd, *J* = 15.6, 5.9, 5.9 Hz, 1H), 4.80 (s, 2H), 4.61 (s, 2H), 4.62 (d, *J* = 10.6 Hz, 1H), 4.57 (d, *J* = 3.7 Hz, 1H), 4.53 (d, *J* = 10.7 Hz, 1H), 4.06 (m, 2H), 4.03-3.94 (m, 2H), 3.85 (ddd, *J* = 10.3, 4.9, 2.4 Hz, 1H), 3.80 (s, 3H), 2.09 (m, 1H), 2.03-1.96 (m, 1H), 1.83-1.77 (m, 2H), 1.60-1.46 (m, 2H), 1.18 (d, *J* = 6.4 Hz, 3H), 1.14 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) 159.4, 158.9, 140.5, 138.1, 131.2, 129.6, 128.6, 128.1, 127.9, 125.8, 114.1, 93.6, 93.3, 78.0, 74.2, 73.4, 71.8, 69.7, 64.1, 55.5, 40.7, 36.1, 28.3, 26.1, 26.0, 20.5, 15.7.



Preparation of ((*E*)-4-((*S*)-2-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy) butyl)-3,4-dihydro-2H-pyran-6-yl)-4-methylpent-2-enyloxy)(*tert*-butyl) dimethylsilane 2.44.[22] To a stirring solution of alcohol **2.43** (713.0 mg, 1.40 mmol, 1.0 equiv) in CH_2Cl_2 (14 mL, 0.1M) in a 50 mL round-bottom flask at 0 °C were added 2,6-lutidine (972 µL, 8.40 mmol, 6.0 equiv) and TBSOTf (802 µL, 3.50 mmol, 2.5 equiv) via syringe. The solution was stirred at 0 °C for 1h, then quenched by the addition of

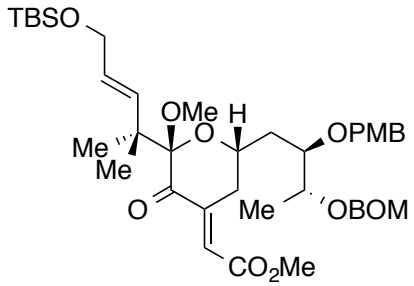
methanol (1 mL). After 5 min at 0 °C, the mixture was transferred to a separatory funnel containing saturated $NaHCO_3$ aqueous solution (10 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 3 × 21 cm silica gel column, eluting with 5% EtOAc/hexanes (600 mL), collecting 9 mL fractions. The fractions containing product (15-27) were combined and concentrated under reduced pressure to give the product **2.44** (768 mg, 88%) as colorless oil: R_f = 0.25 (10% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.34-7.31 (m, 4H), 7.30-7.25 (m, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 5.73 (ddd, *J* = 15.6, 1.5,1.5 Hz, 1H), 5.50 (ddd, *J* = 15.6, 5.4, 5.4 Hz, 1H), 4.85 (ABq, *J* = 6.8 Hz, Δν = 9.3 Hz, 2H), 4.62 (ABq, *J* = 11.7 Hz, Δν = 15.6 Hz, 2H), 4.60 (d, *J* = 10.8 Hz, 1H), 4.53 (dd, *J* = 4.4, 2.9 Hz, 1H), 4.49 (d, *J* = 10.7 Hz, 1H), 4.11 (dd, *J* = 5.4, 1.5 Hz, 2H), 3.99 (dddd, *J* = 9.8, 9.8, 2.4, 2.4 Hz, 1H), 3.93 (ddd, *J* = 11.2, 6.4, 6.4, Hz, 1H), 3.81 (ddd, *J* = 10.3, 2.0, 2.0 Hz, 1H), 3.77 (s, 3H), 2.06 (dddd, *J* = 17.1, 9.8, 6.8, 2.9 Hz, 1H), 1.99-1.91 (m, 1H), 1.80 (ddd, *J* = 14.1, 10.7, 2.4 Hz, 1H), 1.78 (ddd, *J* = 13.2, 7.4, 2.9 Hz, 1H), 1.63 (ddd, *J* = 14.2, 10.8, 2.4 Hz, 1H), 1.47 (dddd, *J* = 13.2, 9.8, 9.8 5.9 Hz, 1H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.15 (s, 3H), 1.14 (s, 3H), 0.88 (s, 9H), 0.03 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) 159.3, 159.1, 138.6, 138.1, 131.1, 129.7, 128.6, 128.0, 127.9, 125.9, 114.0, 93.6, 93.2, 78.0, 74.1, 73.6, 71.7, 69.5, 64.6, 55.5, 40.5, 36.4, 28.3, 26.2, 26.1, 25.9, 20.6, 18.6, 15.8, -4.8.



Preparation of (2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy)butyl)-2-((*E*)-5-(tert-butyldimethylsilyloxy)-2-methylpent-3-en-2-yl)-2-methoxydihydro-2H-pyran-3(4H)-one 2.20.[22] To a stirring solution of dihydropyran **2.44** (70 mg, 0.112 mmol, 1.0 equiv) in CH_2Cl_2 (1.1 mL, 0.1 M) in a 10 mL round-bottom flask at -15 °C was added methanol (500 μL) followed by $NaHCO_3$ (28 mg, 0.34 mmol, 3.0 equiv). The solution was stirred at -15 °C for 5 min, then MMPP (138 mg, 0.28 mmol, 2.5 equiv) was added. The reaction was stirred at -15 °C for 1.5 h, and then warmed to 0 °C and stirred for an additional 30 min. The reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL) followed by saturated aqueous $NaHSO_3$ solution (2 mL). The mixture was stirred at rt for 10 min until effervescence was complete. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried with Na_2SO_4, filtered, and then concentrated under reduced pressure to provide the crude intermediate alcohol as colorless oil, which was carried on to the next step without further purification.

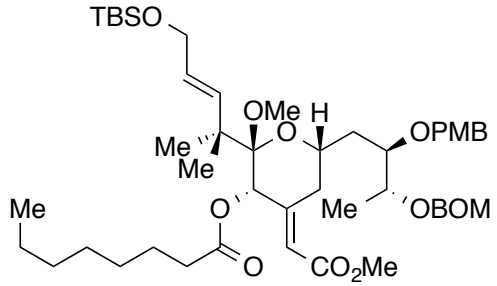
To a stirring solution of the aforementioned crude alcohol (assumed to be 0.112 mmol) in CH_2Cl_2 (1.1 mL) in a 15 mL round-bottom flask at rt were added 4 Å molecular sieves (112 mg), TPAP (4 mg, 0.011 mmol, 0.1 equiv), and NMO (39 mg, 0.336 mmol, 3.0 equiv). The mixture was stirred at rt for 1 h. The reaction was concentrated under N_2 and then directly applied for flash column chromatography on a 3 × 12 cm flash chromatography, eluting with 15% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (11-17) were combined and concentrated under reduced pressure to give the product ketone **2.20** (54 mg, 72% over 2 steps) as colorless oil: R_f = 0.47 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ; 7.39-7.34 (m, 4H), 7.34-7.28

(m, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.98 (dt, *J* = 15.6, 1.5 Hz, 1H), 5.51 (dt, *J* = 16.1, 4.9 Hz, 1H), 4.85 (ABq, *J* = 7.3 Hz, Δν = 7.8 Hz, 2H), 4.67 (s, 2H), 4.63 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 10.8 Hz, 1H), 4.15 (dd, *J* = 4.9, 1.5 Hz, 2H), 3.89 (ddd, *J* = 12.2, 4.9, 2.0 Hz, 1H), 3.81-3.79 (m, 1H), 3.79 (s, 3H), 3.24 (s, 3H), 2.45 (dd, *J* = 5.4, 1.0 Hz, 1H), 2.42 (d, *J* = 5.4 Hz, 1H), 2.00-1.86 (m, 3H), 1.66 (ddd, *J* = 12.9, 10.6, 2.4 Hz, 1H), 1.22 (d, *J* = 6.4 Hz, 3H), 1.44 (s, 3H), 1.10 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$); 207.7, 159.6, 138.0, 136.2, 130.8, 129.4, 128.6, 128.0, 127.9, 127.8, 114.0, 104.3, 93.6, 77.3, 72.5, 72.2, 70.3, 69.7, 64.2, 55.5, 52.3, 44.1, 37.7, 36.4, 30.2, 26.2, 23.0, 22.2, 18.6 14.9, -5.0.



Preparation of (*E*)-methyl 2-((2*S*,6*S*)-6-((2*R*,3*R*)-3-(benzyloxymethoxy)-2-(4-methoxy benzyloxy)butyl)-2-((*E*)-5-(tert-butyldimethylsilyloxy)-2-methylpent-3-en-2-yl)-2-methoxy-3-oxo-2H-pyran-4(3H,5H,6H)-ylidene)acetate 2.45.[22] To a stirring solution of ketone **2.20** (1.6 g, 2.38 mmol, 1.0 equiv) in methanol (24 mL, 0.1 M) at rt was added K_2CO_3 (1.8 g, 13.0 mmol, 5.5 equiv) in one portion. Freshly prepared and distilled methyl glyoxylate (3.7 mL, 47.6 mmol, 20.0 equiv) wad added via syringe. The mixture was stirred at rt for 1 h, and then diluted with Et_2O (10 mL) and quenched by the addition of saturated aqueous NH_4Cl solution (10 mL). The aqueous phase was separated and extracted with Et_2O (3 × 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was

accomplished using flash chromatography on a 3 × 21 cm column, eluting with 10% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (12-29) were combined and concentrated under reduced pressure to give the product **2.45** (1.43 g, 81%) as bright yellow oil: R_f = 0.55 (4:5:1 of hexanes/EtOAc/Et_2O); 500 MHz ^{1}H NMR ($CDCl_3$) δ; 7.40-7.34 (m, 4H), 7.33-7.29 (m, 1H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.54 (dd, *J* = 3.4, 2.0 Hz, 1H), 5.81 (ddd, *J* = 15.6, 1.5, 1.5 Hz, 1H), 5.41 (ddd, *J* = 16.1, 5.4, 5.4 Hz, 1H), 4.85 (ABq, *J* = 6.8 Hz, Δν = 12.2 Hz, 2H), 4.67 (s, 2H), 4.61 (d, *J* = 10. 7 Hz, 1H), 4.41 (d, *J* = 10.7 Hz, 1h), 4.17-4.08 (m, 2H), 4.06 (dd, *J* = 9.3, 2.0 Hz, 1H), 3.90 (ddd, *J* = 10.3, 4.4, 2.0 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.31 (ddd, *J* = 18.6, 2.0, 2.0 Hz, 1H), 3.20 (s, 3H), 2.86 (ddd, *J* = 18.6, 12.2, 3.4 Hz, 1H), 1.97 (ddd, *J* = 14.7, 9.3, 2.0 Hz, 1H), 1.75 (ddd, *J* = 14.7, 9.8, 2.4 Hz, 1H), 1.21 (d, *J* = 6.4 Hz, 3H), 1.10 (s, 3H), 1.04 (s, 3H), 0.90 (s, 9H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$); 197.7, 166.2, 159.3, 148.2, 138.0, 134.8, 130.6, 129.3, 128.7, 128.6, 128.0, 127.9, 122.7, 114.0, 93.6, 76.9, 72.3, 71.7, 69.7, 69.5, 64.1, 55.4, 52.2, 51.9, 44.6, 36.1, 36.1, 26.1, 22.5, 22.0, 14.7, -5.0.

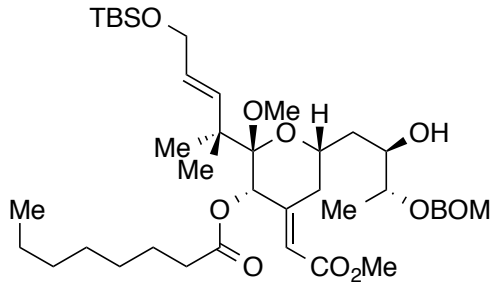


Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-((*E*)-5-((tert-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-3-yloctanoate 2.48.[22] To a stirring solution of ketone **2.45** (409 mg, 0.552 mmol, 1.0 equiv) in methanol (55

mL, 0.01M) in a 100 mL round-bottom flask at rt was added $CeCl_3 \cdot 7H_2O$ (1.64 g, 4.40 mmol, 8 equiv). The reaction mixture was stirred at rt until most of the $CeCl_3 \cdot 7H_2O$ crystals had dissolved, then cooled to -40 °C and stirred for 15 min. $NaBH_4$ (83 mg, 2.20 mmol, 4.0 equiv) was then added in one portion. The mixture was stirred at -40 °C for 1.5 h, and then diluted with 40% EtOAc/hexanes (10 mL), and quenched by the addition of saturated aqueous NH_4Cl solution (5.0 mL). The mixture was poured into a separatory funnel containing 40% EtOAc/hexanes (50 mL). The organic phase was separated, washed with water (10 mL) and brine (10 mL), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude product was used in the next step without further purification.

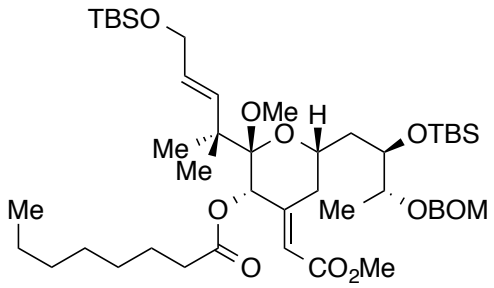
To a stirring solution of the aforementioned crude alcohol (assumed to be 0.552 mmol) in CH_2Cl_2 (5.0 mL, 0.1 M) in a 25 mL round-bottom flask at rt, were added pyridine (446 μL, 5.52 mmol, 10 equiv), DMAP (134 mg, 1.10 mmol, 2.0 equiv), and octanoic anhydride (795 μL, 2.76 mmol, 5.0 equiv). The reaction mixture was stirred at rt overnight, then quenched by the addition of methanol (1.0 mL). The mixture was stirred for another 10 min and 10 mL of CH_2Cl_2 was added. The mixture was poured into a separatory funnel containing 10 mL of saturated aqueous $NaHCO_3$ solution. The aqueous phase was separated and extracted with CH_2Cl_2 (3 × 25 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 3 × 21 cm silica gel column, eluting with 10% EtOAc/hexanes (1000 mL), collecting 9 mL fractions. The fractions containing product (23-59) were combined and concentrated under reduced pressure to give the product **2.48** (388.6 mg, 81%) as colorless oil. R_f = 0.52 (30%

EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.28 (m, 5H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 5.98 (d, *J* = 15.6 Hz, 1H), 5.88 (s, 1H), 5.43 (s, 1H), 5.40 (ddd, *J* = 16.1, 5.3, 5.3 Hz, 1H), 4.86 (ABq, *J* = 6.8 Hz, Δν = 7.0 Hz, 2H), 4.67 (ABq, *J* = 12.2 Hz, Δν = 7.2 Hz, 2H), 4.62 (d, *J* = 10.7 Hz, 1H0, 4.43 (d, *J* = 10.8 Hz, 1H), 4.15-4.03 (m, 3H), 3.90 (ddd, *J* = 10.3, 4.4, 2.0 Hz, 1H), 3.79 (s, 3H), 3.69 (s, 3H), 3.51 (dd, *J* = 15.6, 2.4 Hz, 1H), 3.23 (s, 3H), 2.36 (t, *J* = 7.3 Hz, 2H), 2.29 (ddd, *J* = 7.3, 2.9, 2.9 Hz, 2H), 1.91 (ddd, *J* = 14.2, 9.8, 2.0 Hz, 1H), 1.73 (ddd, *J* = 13.7, 10.3, 2.4 Hz, 1H), 1.64 (d, *J* = 7.3 Hz, 2H), 1.62-1.57 (m, 1H), 1.37-1.26 (m, 10H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 6H), 0.91 (s, 9H), 0.89 (t, *J* = 7.3 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 179.8, 172.3, 166.7, 159.4, 152.7, 138.2, 130.7, 129.5, 128.7, 128.0, 127.9, 124.9, 117.5, 114.0, 102.8, 93.5, 77.0, 72.6, 72.1, 72.0, 69.6, 68.4, 64.7, 55.5, 51.7, 51.3, 45.9, 36.5, 34.5, 34.2, 32.8, 31.9, 29.2, 26.2, 24.9, 24.6, 23.7, 22.8, 18.6, 15.0, 14.3, -4.9.



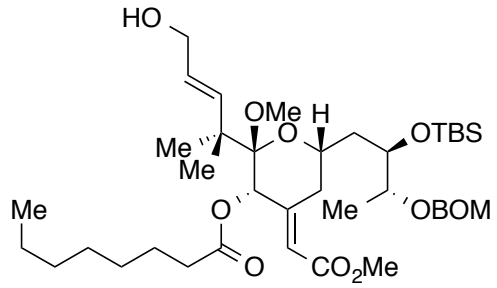
Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-hydroxybutyl)- 2-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 2.49. To a solution of the PMB ether **2.48** (290 mg, 0.335 mmol, 1 equiv) in CH_2Cl_2 (16 mL, 0.02 M) was added *t*BuOH (670 μL) followed by pH 7 buffer (7 mL). The reaction was cooled to 0 ^{o}C. DDQ (228 mg, 1.005 mmol) was then added to the reaction and vigorously stirred for 1 h at rt. The reaction was then quenched with saturated aqueous

$NaHCO_3$ solution. The phases separated and the organic layer was extracted with CH_2Cl_2 (3 × 20 mL) and washed with brine (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 3 × 21 cm silica gel column, eluting with 10% EtOAc/hexanes (200 mL), 25% EtOAc/hexanes (400 mL), collecting 9 mL fractions. The fractions containing product (48-65) were combined and concentrated under reduced pressure to give the product **2.49** (218 mg, 87%) as colorless oil. R_f = 0.42 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = -3.2 (*c* = 0.120, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.34 (m, 4H), 7.33-7.28 (m, 1H), 6.01 (d, *J* = 15.6 Hz, 1H), 5.88 (s, 1H), 5.42 (s, 1H), 5.36 (ddd, *J* = 16.1, 5.4, 5.4 Hz, 1H), 4.87 (ABq, *J* = 6.8 Hz, Δν = 26.7 Hz, 2H), 4.66 (ABq, *J* = 11.7 Hz, Δν = 19.5 Hz, 2H), 4.16 (ddd, *J* = 8.8, 8.8, 2.9 Hz, 1H), 4.12 (dd, *J* = 4.9, 1.5 Hz, 2H), 3.87 (m, 1H), 3.68 (s, 3H), 3.63 (m, 1H), 3.50 (m, 1H), 3.36 (s, 3H), 2.69 (m, 1H), 2.36-2.24 (m, 3H), 1.72 (m, 2H), 1.59 (m, 2H), 1.32-1.22 (m, 12H), 1.12 (s, 3H), 1.11 (s, 3H), 0.91 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.2, 166.7, 152.8, 138.2, 137.6, 128.6, 128.0, 128.0, 124.4, 117.3, 102.8, 93.9, 78.1, 72.2, 71.2, 70.0, 68.3, 64.5, 51.6, 51.3, 45.9, 39.8, 34.5, 32.6, 31.9, 31.8, 29.2, 26.2, 24.8, 24.6, 23.6, 22.8, 18.6, 18.6, 16.9, 14.3, -4.9; IR (neat) 2951, 2928, 2845, 1746, 1722, 1627, 1512, 1453, 1435, 1380, 1310, 1247, 1228, 1151, 1110, 1071, 1039, 902, 836, 690 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{41}H_{68}O_{10}SiNa$ ($M + Na^+$) 771.4474, found 771.4482.



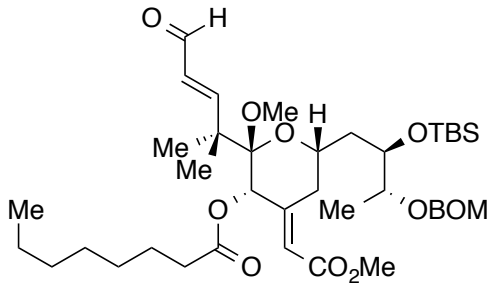
Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 2.50. A solution of the alcohol **2.49** (154.8 mg, 0.207 mmol, 1 equiv) in CH_2Cl_2 (4 mL, 0.05 M) in a 15 mL round-bottom flask was cooled to 0 ^oC. To this solution was added 2,6-lutidine (144 μL, 1.24 mmol, 6 equiv) followed by TBSOTf (119 μL, 0.516 mmol, 2.5 equiv) via syringe. The reaction was stirred for 45 min, after which the reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (2 mL). The phases separated and the organic layer was extracted with CH_2Cl_2 (3 × 20 mL), then washed with brine (20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1 × 10 cm silica gel column, eluting with 5% EtOAc/hexanes (200 mL), collecting 9 mL fractions. The fractions containing product (12-20) were combined and concentrated under reduced pressure to give the product **2.50** (164.1 mg, 92%) as colorless oil: R_f = 0.62 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = -13.2 (*c* = 0.200, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.32 (m, 4H), 7.32-7.25 (m, 1H), 5.95 (d, *J* = 16.1 Hz, 1H), 5.90 (s, 1H), 5.50 (s, 1H), 5.40 (ddd, *J* = 16.1, 5.4, 5.4 Hz, 1H), 4.80 (s, 2H), 4.64 (ABq, *J* = 11.7 Hz, Δν = 13.6 Hz, 2H), 4.10 (dd, *J* = 5.4, 1.4 Hz, 2H), 4.05 (ddd, *J* = 8.8, 8.8, 2.9 Hz, 2H), 3.85 (ddd, *J* = 10.7, 6.3, 6.3 Hz, 1H), 3.69 (s, 3H), 3.52

(dd, *J* = 15.6, 2.4 Hz, 1H), 3.32 (s, 3H), 2.30 (m, 3H), 1.99 (ddd, *J* = 11.2, 8.8, 1.9 Hz, 1H), 1.60 (s, 3H), 1.29 (m, 9H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.11 (s, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.3, 166.6, 152.9, 138.0, 137.9, 128.6, 127.9, 127.8, 125.3, 117.3, 102.6, 93.2, 75.0, 71.6, 70.2, 69.5, 68.5, 64.7, 51.9, 51.3, 45.9, 38.7, 34.6, 33.0, 31.9, 29.2, 29.1, 26.2, 26.1, 24.9, 24.6, 23.7, 22.8, 18.6, 18.3, 14.3, 13.9, -3.9, -4.5, -4.9; IR (neat) 2951, 2943, 2928, 2845, 1746, 1722, 1627, 1512, 1453, 1443, 1381, 1247, 1228, 1151, 1110, 1071, 1039, 902, 836, 812 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{47}H_{82}O_{10}Si_2Na$ (M + Na^+) 885.5339. Found 885.5351.



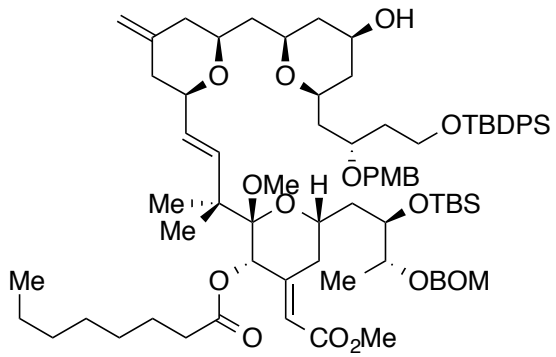
Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-5-hydroxy-2-methylpent-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 2.51. To a solution of the TBS ether **2.50** (27 mg, 0.031 mmol, 1 equiv) in THF (156 μL, 0.2 M) in a 10 mL plastic centrifuge vial was added pyridine (31 μL) followed by HF•Py (28 μL, 20% HF basis, 0.312 mmol, 10 equiv) via plastic pipette. The reaction was stirred for 4 h at rt, after which the reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (2 mL). The phases separated and the organic layer was extracted with CH_2Cl_2 (3 × 10 mL), then washed with brine (10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification was

accomplished by flash column chromatography on a 1 × 10 cm silica gel column, eluting with 25% EtOAc/hexanes (200 mL), collecting 9 mL fractions. The fractions containing product (8-12) were combined and concentrated under reduced pressure to give the product **2.51** (20.4 mg, 87%) as colorless oil. R_f = 0.32 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = -1.2 (*c* = 0.120, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.33 (m, 4H), 7.38-7.28 (m, 1H), 5.97 (d, *J* = Hz, 1H), 5.89 (s, 1H), 5.57 (s, 1H), 5.51 (ddd, *J* = 16.1, 5.4, 5.4 Hz, 1H), 4.81 (s, 2H), 4.65 (s, 2H), 4.15-4.10 (m, 4H), 3.86 (ddd, *J* = 8.8, 8.8, 2.9 Hz, 2H), 3.70 (s, 3H), 3.46 (dd, *J* = 16.1, 1.9 Hz, 1H), 3.34 (s, 3H), 2.42-2.33 (m, 1H), 2.34-2.27 (ddd, J = 7.8, 7.8, 3.9 Hz, 2H), 1.99 (ddd, *J* = 11.2, 8.8, 1.9 Hz, 1H), 1.67-1.56 (m, 4H), 1.29 (m, 9H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 0.90 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.5, 166.8, 152.9, 139.7, 138.0, 128.6, 128.0, 127.9, 125.5, 117.1, 102.6, 93.3, 75.1, 71.5, 70.3, 69.5, 68.6, 64.3, 51.7, 51.4, 46.2, 38.7, 34.7, 33.4, 31.9, 29.2, 29.1, 26.1, 24.2, 24.1, 22.8, 18.3, 14.3, 13.9, -3.8, -4.5; IR (neat) 2933, 2940, 2857, 1746, 1722, 1689, 1627, 1512, 1463, 1442, 1437, 1380, 1300, 1243, 1228, 1156, 1110, 1071, 1043, 912, 836, 773, 736, 698, 505 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{41}H_{68}O_{10}SiNa$ (M + Na^+) 771.4474. Found 771.4484.



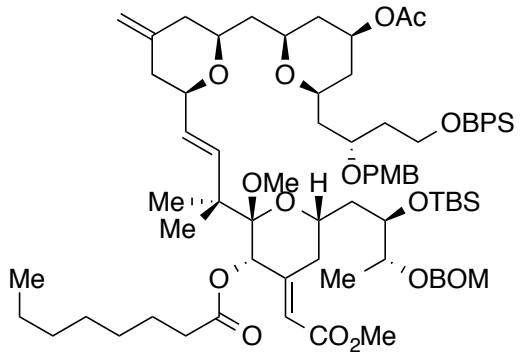
Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)tetrahydro-2*H*-pyran-3-yl octanoate 2.11.[22] To a stirring

solution of the alcohol **2.51** (17.1 mg, 0.023 mmol, 1.0 equiv) in CH_2Cl_2 (226 μL, 0.1 M) and *t*BuOH (2.5 μL) in a 5 mL vial at 0 °C was added pyridine (5.5 μL, 0.068 mmol, 3 equiv) followed by DMP (14 mg, 0.034 mmol, 1.5 equiv). Stirring was continued at 0 °C for 2 h. The reaction mixture was then quenched by the addition of saturated aqueous $Na_2S_2O_3$ solution (2 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 1 × 10 cm silica gel column, eluting with 10% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (10-14) were combined and concentrated under reduced pressure to provide the product aldehyde **2.11** (16.1 mg, 95%) as colorless oil: R_f = 0.63 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.45 (d, *J* = 7.8 Hz, 1H), 7.30-7.20 (m, 5H), 5.87 (dd, *J* = 16.1, 7.8 Hz, 1H), 5.84 (s, 1H), 5.48 (s, 1H), 4.74 (ABq, *J* = 6.8 Hz, Δν = 6.3 Hz, 2H), 4.57 (s, 2H), 4.15-4.08 (m, 2H), 3.87 (dd, *J* = 6.4, 4.4 Hz, 1H), 3.64 (s, 3H), 3.49 (dd, *J* = 16.1, 2.0 Hz, 1H), 3.30 (s, 3H), 2.30 (dd, *J* = 14.2, 13.7 Hz, 1H), 2.13 (ddd, *J* = 16.1, 7.3, 7.3 Hz, 1H), 2.04 (ddd, *J* = 16.1, 7.3, 7.3 Hz, 1H), 1.98 (ddd, *J* = 14.2, 8.8, 2.4 Hz, 1H), 1.59 (ddd, *J* = 14.2, 8.8, 2.9 Hz, 1H), 1.51-1.44 (m, 1H), 1.25-1.15 (m, 10H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.11 (s, 3H), 1.08 (s, 3H), 0.82 (s, 9H), 0.80 (t, J = 6.8 Hz, 3H), 0.03 (s, 3H), 0.0 (s, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.8, 172.1, 167.1, 166.4, 151.8, 137.9, 128.7, 128.0, 127.3, 117.9, 102.6, 93.3, 75.1, 70.8, 70.3, 69.6, 69.2, 51.9, 51.5, 47.6, 38.7, 34.6, 33.2, 31.8, 29.1, 26.1, 24.8, 23.7, 22.8, 22.1, 18.3, 14.3, 13.9, -3.8, -4.5.



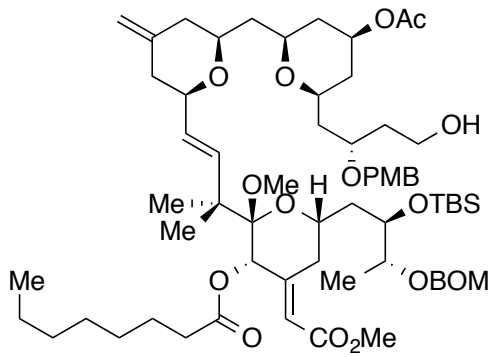
Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*R*,4*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-4-hydroxytetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 2.52.[22] To a stirring solution of hydroxyallylsilane **2.12** (13 mg, 0.018 mmol, 1.3 equiv) and aldehyde **2.11** (10.5 mg, 0.014 mmol, 1.0 equiv) in Et_2O (3 mL, 0.005 M) in a 15 mL round-bottom flask at -78 ºC was added a solution of TMSOTf in Et_2O (18 μL, 1.0 M in Et_2O, 0.018 mmol, 1.3 equiv) dropwise via syringe. After 1 h at -78 ºC, the mixture was slowly warmed to -40 ºC and stirred for 2 h, and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (2 mL). The mixture was warmed to rt, and then the phases were separated, and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 0.5 × 10 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product (8-13) were combined and concentrated under reduced pressure to give the product **2.52** (12.9 mg, 67% yield) as colorless oil. R_f = 0.49 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) 7.72-7.64 (m, 4H), 7.46-7.28 (m, 11H),

7.18 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 2H), 5.94 (s, 1H), 5.90 (d, *J* = 16.1 Hz, 1H), 5.61 (s, 1H), 5.42 (dd, *J* = 16.1, 5.9 Hz, 1H), 4.79 (s, 2H), 4.66-4.59 (m, 2H), 4.64 (s, 2H), 4.40 (ABq, *J* = 10.7 Hz, Δν = 37.6 Hz, 2H), 4.12-4.06 (m, 2H), 3.93-3.89 (m, 1H), 3.88 -3.72 (m, 5H), 3.80 (s, 3H), 3.68 (s, 3H), 3.60-3.47 (m, 4H), 3.31 (s, 3H), 2.40-2.28 (m, 3H), 2.24 (d, *J* = 13.2 Hz, 1H), 2.16 (d, *J* = 13.7 Hz, 1H), 2.02-1.85 (m, 6H), 1.84 - 1.73 (m, 3H), 1.67-1.50 (m, 6H), 1.32-1.22 (m, 10H), 1.16 (d, *J* = 6.4 Hz, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.06 (s, 9H), 0.90-0.85 (m, 3H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); 13C NMR 125 MHz ($CDCl_3$) δ 172.3, 166.6, 159.3, 153,3, 144.4, 138.0, 137.9, 135.8, 134.1, 134.0, 131.2, 129.8, 129.5, 128.6, 128.0, 127.8, 127.6, 116.6, 114.0, 109.0, 102.6, 93.2, 79.2, 75.0, 72.7, 72.0, 71.9, 71.5, 70.2, 69.5, 68.4, 60.6, 55.5, 51.5, 51.3, 46.2, 42.5, 42.4, 41.8, 41.3, 41.0, 40.5, 38.7, 37.9, 34.6, 33.8, 31.9, 29.3, 29.2, 27.2, 26.1, 25.0, 24.4, 24.0, 22.8, 19.4, 18.3, 14.3, 14.0, -3.8, -4.4.



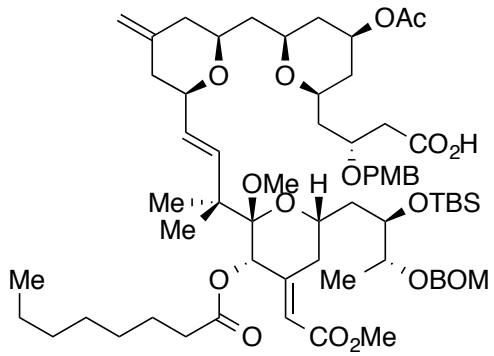
Preparation of (2*S*,3*S*,6*S*,*E*)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*R*)-4-acetoxy-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)tetrahydro-2H-pyran-2-yl)methyl)-4-methylenetetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-3-yl octanoate 2.53.[22] To a stirring solution of the alcohol **2.52** (195 mg, 0.142 mmol, 1 equiv) in CH_2Cl_2 (14 mL, 0.01 M) in

a 25 mL round-bottom flask at rt were added pyridine (573 μL, 7.08 mmol, 50 equiv), DMAP (173 mg, 1.42 mmol, 10 equiv) and Ac_2O (401 μL, 4.25 mmol, 30 equiv). The mixture was stirred at rt overnight, and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography with a 3 × 21 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 9 mL fractions. The fractions containing product (14-23) were combined and concentrated under reduced pressure to provide the product **2.53** (170.8 mg, 85%) as a colorless oil: R_f = 0.49 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.64 (m, 4H), 7.46-7.26 (m, 11H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.93 (d, *J* = 16.1 Hz, 1H), 5.90 (s, 1H), 5.58 (s, 1H), 5.42 (dd, *J* = 16.1, 5.4 Hz, 1H), 4.80 (s, 2H), 4.65-4.59 (m, 3H), 4.53 (bs, 1H), 4.44 (d, *J* = 10.7 Hz, 1H), 4.34 (d, *J* = 10.7 Hz, 1H), 4.11-4.05 (m, 2H), 3.91-3.82 9m, 2H), 3.80 (s, 3H), 3.79-3.72 (m, 2h), 3.68 (s, 3H), 3.67-3.60 (m, 2H), 3.58-3.45 (m, 3H), 3.30 (s, 3H), 2.38-2.30 (m, 3H), 2.26 (d, *J* = 12.7 Hz, 1H), 2.17 (d, *J* = 14.7 Hz, 1H), 2.04 (s, 3H), 2.06-1.95 (m, 3H), 1.92-1.85 (m, 2H), 1.81-1.73 (m, 3H), 1.66-1.54 (m, 6H), 1.33-1.23 (m, 10H), 1.16 (d, *J* = 6.4 Hz, 3H), 1.11 (s, 6H), 1.05 (s, 9H), 0.90-0.85 (m, 3H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); 13C NMR 125 MHz ($CDCl_3$) δ 172.4, 170.6, 166.7, 159.4, 153,1, 144.2, 138.1, 138.1, 135.8, 134.1, 134.0, 131.2, 129.8, 129.5, 128.6, 128.0, 127.9, 127.3, 117.0, 114.1, 109.1, 102.6, 93.3, 79.0, 75.0, 74.9, 72.8, 72.0, 71.8, 71., 70.7, 70.3, 69.5, 68.4, 60.6, 55.5, 51.6, 51.3, 46.1, 42.8, 42.3, 40.8, 40.4, 38.7, 37.9, 37.9, 37.8, 34.6, 33.5, 31.9, 29.3, 29.2, 27.1, 26.1, 24.9, 24.2, 24.1, 22.8, 21.5, 19.4, 18.3, 14.0, -3.9, -4.4.



Preparation of (2*S*,3*S*,6*S*,*E*)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,4*S*,6*R*)-4-acetoxy-6-((*S*)-4-hydroxy-2-((4-methoxybenzyl)oxy)butyl)tetrahydro-2H-pyran-2-yl)methyl)-4-methylene tetrahydro-2H-pyran-2-yl)-2-methylbut-3-en-2-yl)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2H-pyran-3-yl octanoate 2.54.[22] To a stirring solution of BPS ether **2.53** (32.2 mg, 0.023 mmol, 1.0 equiv) in DMF (2 mL, 0.01 M) in a 15 mL round-bottom flask were added a solution of TBAF solution in THF (45 μL, 1.0 M in THF, 0.045 mmol, 2.0 equiv) and AcOH (45 μL, 1.0 M in DMF, 0.045 mmol, 2.0 equiv). The solution was stirred at rt for 2 days, and then diluted with 40% EtOAc/hexanes (100 mL) and water (5 mL). The phases were separated and the organic phase was washed with water (3 × 10 mL). The organic phases were dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 2 × 15 cm silica gel column, eluting with 30% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product (26-41) were combined and concentrated under reduced pressure to provide the alcohol **2.54** (22.2 mg, 83%) as colorless oil. R_f = 0.33 (30% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.38-7.28 (m, 5H), 7.26 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 5.94 (d, *J* = 15.6 Hz, 1H), 5.89 (s, 1H), 5.57 (s, 1H), 5.41 (dd, *J* = 16.1, 5.9 Hz, 1H), 4.95-4.88 (m, 1H), 4.80 (s,

2H), 4.68-4.57 (m, 2H), 4.64 (s, 2H), 4.47 (ABq, J = 10.7 Hz, Δν = 33.5 Hz, 2H), 4.12-4.05 (m, 3H), 3.90-3.83 (m, 3H), 3.81 (s, 3H), 3.76-3.70 (m, 2H), 3.69 (s, 3H), 3.62-3.45 (m, 4H), 3.30 (s, 3H), 2.39-2.30 (m, 1H), 2.34 (td, J = 7.3, 2.4 Hz, 2H), 2.25 (d, J = 13.2 Hz, 1H), 2.18 (d, J = 12.7 Hz, 1H), 2.04 (s, 3H), 2.04-1.88 (m, 7H), 1.80-1.70 (m, 2H), 1.70-1.52 (m, 6H), 1.33-1.23 (m, 10H), 1.16 (d, J = 6.4 Hz, 3H), 1.10 (s, 6H), 0.90-0.85 (m, 3H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR 125 MHz ($CDCl_3$) δ 172.4, 170.7, 166.7, 159.6, 153.1, 144.2, 138.4, 138.1, 130.5, 129.7, 128.6, 128.0, 127.9, 127.2, 117.0, 114.2, 109.1, 102.6, 93.2, 79.3, 75.2, 75.0, 75.0, 72.4, 72.2, 72.0, 71.5, 70.5, 70.2, 69.5, 68.4, 60.3, 55.5, 51.6, 51.3, 46.1, 42.7, 41.6, 40.8, 40.3, 38.6, 38.0, 37.6, 36.8, 34.6, 33.5, 31.9, 29.3, 29.2, 26.1, 24.9, 24.2, 24.1, 22.8, 21.5, 18.3, 14.3, 14.0, -3.8, -4.5.

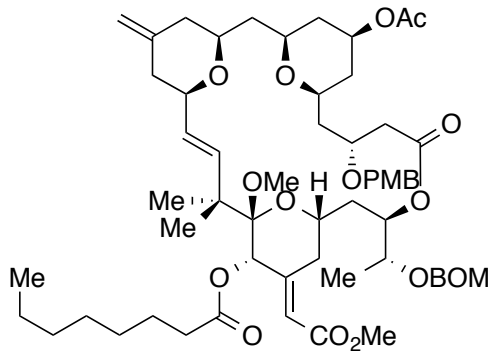


Preparation of (*R*)-4-((2*R*,4*S*,6*S*)-4-acetoxy-6-(((2*S*,6*R*)-6-((*E*)-3-((2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-3-(octanoyloxy)tetrahydro-2H-pyran-2-yl)-3-methylbut-1-en-1-yl)-4-methylenetetrahydro-2H-pyran-2-yl)methyl)tetrahydro-2H-pyran-2-yl)-3-((4-methoxybenzyl)oxy)butanoic acid 2.56.[22] To a stirring solution of alcohol **2.54** (89.7 mg, 0.076 mmol, 1.0 equiv) in CH_2Cl_2 (7.6 mL, 0.01M) in a 25 mL round-bottom flask at -10 °C were added diisopropylethylamine (185 μL, 1.065 mmol, 14.0 equiv) and then dimethyl sulfoxide (108 μL, 1.52 mmol, 20.0 equiv). The solution

was stirred at -10 °C for 5 min and SO_3·Py (97 mg, 0.608 mmol, 8.0 equiv) was added in one portion. Stirring was continued at -10 °C for 1 h and then warmed up to 0 oC and stirred for another 30 min, after which the reaction mixture was diluted with CH_2Cl_2 (1 mL) and quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The mixture was stirred at rt for 10 min until effervescence was complete. The reaction mixture was partitioned between CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ solution (20 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was washed through a small plug of silica gel with 20% EtOAc/hexanes (100 mL), and the solvent was removed under reduced pressure to provide the aldehyde, which was used in the next step without further purification.

To a stirring solution of the aforementioned aldehyde (assumed to be 0.076 mmol) in 2-methyl-2-butene (2 mL, 0.04 M) and *t*BuOH (4 mL) in a 25 round-bottom flask at rt was added aqueous solution of KH_2PO_4 (762 μL, 1.0 M in H_2O). The mixture was cooled to 0 °C, and $NaClO_2$ (70.0 mg, 0.760 mmol, 20.0 equiv) was added in one portion. The reaction mixture was stirred vigorously at 0 °C for 1.5 h, and then quenched by the addition of aqueous pH 4 buffer solution (5 mL). The resulting mixture was partitioned between CH_2Cl_2 (25 mL) and aqueous pH 4 buffer solution (5 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases were dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished using flash chromatography with a 1 × 10 cm silica gel column, eluting with 5% MeOH/20% EtOAc/75% hexanes, collecting 9 mL

fractions. The fractions containing product (23-45) were combined and concentrated under reduced pressure to provide the product carboxylic acid **2.56** (73.5 mg, 81% over 2 steps) as colorless oil: R_f = 0.12 (5% MeOH/20% EtOAc/75% hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.37-7.28 (m, 5H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.96 (d, *J* = 16.1 Hz, 1H), 5.89 (s, 1H), 5.58 (s, 1H), 5.45 (dd, *J* = 15.6, 6.3 Hz, 1H), 4.90 (m, 1H), 4.83-4.74 (m, 1H), 4.81 (s, 2H), 4.70-4.59 (m, 4H), 4.59-4.40 (m, 2H), 4.10 (m, 5H), 3.89-3.82 (m, 2H), 3.88-3.83 (m, 6H), 3.80 (s, 3H), 3.80-3.70 (m, 2H), 3.69 (s, 3H), 3.63-3.44 (m, 4H), 3.31 (s, 3H), 2.61 (d, *J* = 5.9 Hz, 2H), 2.40-2.30 (m, 2H), 2.25 (d, *J* = 12.7 Hz, 1H), 2.17 (d, *J* = 13.2 Hz, 1H), 2.07-1.90 (m, 7H), 2.03 (s, 3H), 1.77-1.55 (m, 7H), 1.34-1.22 (m, 10H), 1.16 (d, *J* = 6.8 Hz, 3H), 1.11 (s, 6H), 0.90-0.85 (m, 3H), 0.88 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.7, 172.4, 170.6, 166.7, 159.6, 153.1, 144.2, 138.5, 138.0, 137.4, 130.3, 129.6, 128.6, 127.9, 127.2, 116.7, 114.1, 109.1, 102.6, 93.1, 79.4, 75.1, 72.9, 72.4, 72.0, 71.6, 70.4, 70.4, 70.2, 69.5, 68.4, 55.5, 51.6, 51.3, 51.0, 46.1, 42.7, 41.9, 40.4, 40.1, 38.6, 37.8, 37.6, 34.6, 31.9, 31.8, 29.2, 26.1, 26.0, 24.9, 24.3, 24.0, 22.8, 21.5, 18.3, 14.3, 13.9, -3.8, -4.3, -4.5; 125 MHz DEPT NMR ($CDCl_3$) CH_3 δ 55.5, 51.4, 51.0, 26.1, 24.3, 24.0, 21.5, 14.3, 14.0, -3.8, -4.5; CH_2 δ 108.9, 92.9, 72.2, 69.3, 41.7, 41.6, 40.6, 40.2, 40.1, 38.6, 37.8, 37.6, 34.6, 34.3, 31.7, 29.0, 28.9, 24.7, 22.6, 22.5; CH δ 138.7, 129.7, 128.6, 128.1, 127.9, 127.2, 116.9, 113.9, 79.4, 75.2, 75.1, 75.0, 73.0, 72.0, 71.6, 70.4, 70.1, 68.2.

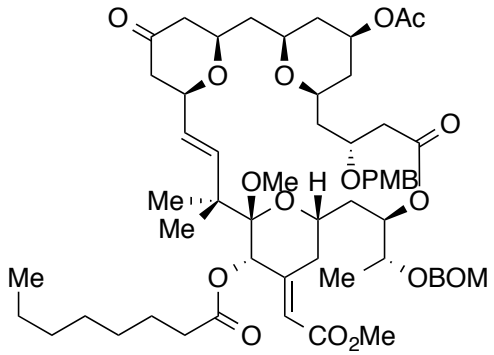


Preparation of (1*S*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5-methylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 2.57. To a stirring solution of TBS ether **2.56** (88.7 mg, 0.074 mmol, 1.0 equiv) in 9:1 THF/pyridine (7.5 mL, 0.01 M) in a 25 mL plastic vial were added methanol (743 μL) and HF·Py (3.9 mL, 20% HF basis). The solution was stirred at rt for 2 d, and then diluted with 50% EtOAc/hexanes (10 mL), and washed with brine (2 × 10 mL). The solution was dried over Na_2SO_4 and concentrated under reduced pressure. The crude seco acid was taken on to the next step without purification.

To a stirring solution of aforementioned seco acid (assumed to be 0.074 mmol) in THF (2.5 mL) in a 50 mL round-bottom flask at 0 °C were added Et_3N (61.7 μL, 0.444 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (34.7 μL, 0.222 mmol, 3.0 equiv). After 5 min, the mixture was warmed to rt and stirring was continued for an additional 3 h. The reaction mixture was diluted with 3:1 toluene/THF (29.6 mL, 0.0025 M) and placed into a 25 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (181 mg, 1.48 mmol, 20.0 equiv) in toluene (49.3 mL, 0.0015 M) preheated at 40 °C over 12 h. The rest of the contents in the flask were transferred

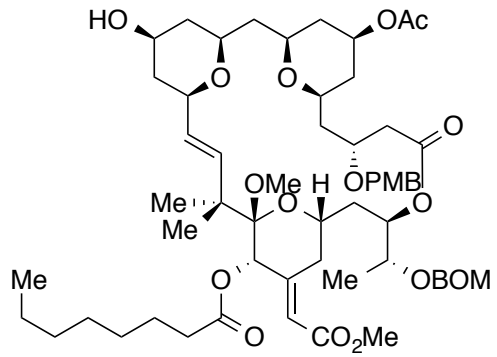
over another 5 h. The residual contents of the syringe were rinsed into the flask with toluene (2 × 1.0 mL) and stirring was continued for an additional 2 h. The reaction mixture was then cooled to rt, diluted with 40% EtOAc/hexanes (100 mL), and washed with water (3 × 30 mL) and with brine (50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography with a 1 × 10 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 9 mL fractions. The fractions containing product (23-47) were combined and concentrated under reduced pressure to provide pure macrolactone **2.57** (48.9 mg, 62% over 2 steps) as colorless oil: R_f = 0.49 (40% EtOAc/hexanes); $[\alpha]^{20}_D$ = +16.5 (*c* = 2.2, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.28 (m, 5H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.22 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.5 Hz, 1H), 5.61-5.55 (m, 1H), 5.34 (dd, *J* = 15.6, 8.8 Hz, 1H), 5.18 (s, 1H), 4.81-4.71 (m, 6H), 4.64 (ABq, *J* = 11.7, Δν = 14.4 Hz, 2H), 4.49 (ABq, *J* = 11.2 Hz, , Δν = 7.0 Hz, 2H), 4.19 (m, 1H), 3.99-3.91 (m, 2H), 3.80-3.73 (m, 1H), 3.76 (s, 3H), 3.73-3.63 (m, 3H), 3.69 (s, 3H), 3.52-3.40 (m, 2H), 3.18 (m, 1H), 3.08 (m, 3H), Hz, 1H), 2.5 (m, 2H), 2.35-2.18 (m, 4H), 2.14-1.83 (m, 10H), 2.03 (s, 3H), 1.80 (m, 2H), 1.75-1.47 (m, 2H), 1.46-1.19 (m, 6H), 1.13-1.04 (m, 3H), 0.88 (t, *J* = 6.8 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.2, 172.1, 170.7, 166.9, 159.3, 151.5, 144.4, 141.7, 138.0, 130.9, 129.6, 128.6, 128.5, 128.0, 127.8, 125.7, 119.4, 113.9, 109.1, 103.3, 93.7, 81.4, 76.3, 74.8, 73.7, 73.5, 73.3, 73.2, 72.2, 70.8, 70.4, 69.8, 67.3, 55.5, 52.7, 51.3, 51.3, 45.2, 43.9, 42.9, 41.9, 41.4, 40.9, 37.6, 34.8, 34.8, 31.8, 31.0, 29.2, 29.0, 26.4, 24.8, 22.8, 21.5, 20.1, 15.3, 14.3; 125 MHz DEPT NMR ($CDCl_3$) CH_3 δ 55.5, 52.7, 51.4, 26.4, 21.5, 20.1, 15.4, 14.3; CH_2 δ 93.7, 71.8, 69.9, 48.9, 48.2, 43.9, 42.9, 41.4, 37.6, 35.0, 34.8, 31.8, 31.0,

29.2 (×2), 29.1, 24.9, 22.8; CH δ 141.7, 129.6, 128.6, 128.0, 127.8, 125.7, 119.4, 113.9, 81.4, 76.3, 74.8, 73.7, 73.5, 73.3, 73.2, 72.5, 70.8, 70.4, 67.3; IR (neat): 2930, 2361, 2339, 1736, 1719, 1654, 1514, 1436, 1175, 1042, 902, 812, 505; HRMS (ESI/TOF) calcd for $C_{60}H_{84}O_{16}Na$ ($M + Na^+$) 1083.5651. Found: 1083.5670.



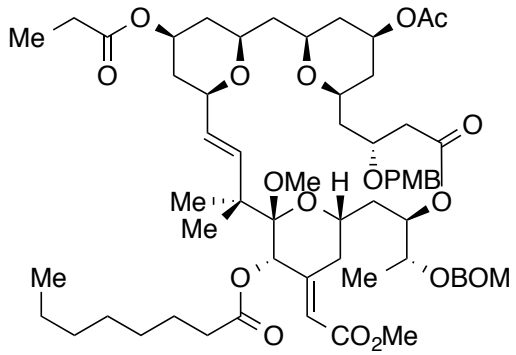
Preparation of (1*S*,3*R*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-5,19-dioxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 2.58.[22] A 100 ml flask containing 50 mL of CH_2Cl_2 was cooled to -78 °C, and a stream of O_3 was passed in for 3 min. The color of the solution changed to light blue. The flask was sealed and kept at -78 °C for immediate use. This O_3 solution was added in 100 μL portion via a plastic syringe to a stirring solution of olefin **2.57** (39.0 mg, 0.037 mmol, 1.0 equiv) at -78 °C. The reaction was monitored by TLC and the addition of the O_3 solution was continued every 10 min until the starting material was fully consumed. DMS (1.2 mL) was then added and the mixture was warmed to rt. The solution was stirred at rt for 12 h, after which the solvent was removed under reduced pressure. Purification was accomplished using flash chromatography on a 1 × 10 cm silica gel column, eluting with 25% EtOAc/hexanes (500 mL), collecting 9 mL fractions. The fractions containing product

(36-64) were combined and concentrated under reduced pressure to provide ketone **2.58** (29.3 mg, 75%) as colorless oil: R_f = 0.49 (40% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.41-7.29 (m, 5H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.5 Hz, 1H), 5.59-5.53 (m, 1H), 5.33 (dd, *J* = 15.6, 8.8 Hz, 1H), 5.19 (s, 1H), 4.80 (s, 2H), 4.62 (ABq, *J* = 11.7, Δν = 14.4 Hz, 2H), 4.42 (ABq, *J* = 11.2 Hz, Δν = 7.0 Hz, 2H), 42.9-4.22 (m, 1H), 4.16-4.10 (m, 1H), 3.90 (dd, *J* = 6.4, 4.4 Hz, 1H), 3.77-3.74 (m, 1H), 3.76 (s, 3H), 3.70-3.65 (m, 3H), 3.67 (s, 3H), 3.48-3.40 (m, 1H), 3.14-3.08 (m, 1H), 3.06 (s, 3H), 2.50-2.40 (m, 2H), 2.35-2.18 (m, 4H), 2.16-2.06 (m, 2H), 2.02 (s, 3H), 1.96-1.76 (m, 5H), 1.64-1.50 (m, 6H), 1.34-1.22 (m, 10H), 1.10-1.06 (m, 9H), 0.86 (t, *J* = 6.3 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 206.8, 172.3, 172.2, 170.7, 167.0, 159.5, 151.3, 142.7, 138.1, 130.6, 129.8, 128.7, 128.0, 127.9, 124.3, 119.6, 114.0, 103.3, 93.7, 79.8, 77.4, 74.5, 74.4, 73.6, 73.4, 72.7, 71.8, 70.9, 70.2, 69.9, 67.3, 55.5, 52.7, 51.4, 48.9, 48.2, 45.3, 43.9, 42.9, 41.4, 37.6, 35.0, 34.8, 31.8, 31.0, 29.2, 29.2, 29.1, 26.4, 24.9, 22.8, 21.5, 20.1, 15.4, 14.3; 125 MHz DEPT NMR ($CDCl_3$) CH_3 δ 55.5, 52.7, 51.4, 26.4, 21.5, 20.1, 15.4, 14.3; CH_2 δ 93.7, 71.8, 69.9, 48.9, 48.2, 43.9, 42.9, 41.4, 37.6, 35.0, 34.8, 31.8, 31.0, 29.2, 29.2, 29.1, 24.9, 22.8; CH δ 142.7, 129.8, 128.7, 128.0, 127.9, 124.3, 119.6, 114.0, 79.8, 77.4, 74.5, 74.4, 73.6, 73.4, 72.7, 70.9, 70.2, 67.3.



Preparation of (1*S*,3*S*,5*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-5-hydroxy-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 2.59. To a solution of the ketone **2.58** (7.7 mg, 0.007 mmol, 1 equiv) in MeOH (724 μL, 0.01 M) at -40 ^{o}C was added $NaBH_4$ (1 mg, 0.029 mmol, 4 equiv). The reaction mixture was stirred for 1.5 h and then quenched by the addition of acetone (100 μL). The reaction was diluted with CH_2Cl_2 (5 mL) and then washed with a saturated NH_4Cl solution (10 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification was accomplished using flash chromatography with a 0.5 × 10 cm silica gel column, eluting with 50% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product (25-30) were combined and concentrated under reduced pressure to provide the product **2.59** (6.0 mg, 78%) as colorless oil: R_f = 0.12 (5% methanol/20% EtOAc/75% hexanes); $[\alpha]^{20}_{D}$ = +19.0 (*c* = 0.510, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.28 (m, 5H), 7.22 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.21 (d, *J* = 15.6 Hz, 1H), 5.95 (d, *J* = 1.9 Hz, 1H), 5.57 (ddd, *J* = 11.7, 4.4, 2.4 Hz, 1H), 5.32 (dd, *J* = 15.6, 8.8 Hz, 1H), 5.18 (s, 1H), 4.89-4.78 (m, 1H), 4.83 (ABq, *J* = 6.8 Hz, Δν = 11.2 Hz, 2H), 4.65 (ABq, *J* = 11.7 Hz, Δν = 18.6 Hz, 2H), 4.48 (ABq, *J* = 10.7, Δν = 13.7 Hz 2H), 4.20-4.13 (m, 1H), 4.00-3.83 (m, 4H), 3.78-3.75 (m, 1H), 3.76 (s, 3H), 3.73-3.71 (m, 1H), 3.71-3.65 (m, 2H), 3.69 (s, 3H), 3.52-3.44 (m, 3H), 3.18 (m, 1H), 3.06 (s, 3H), 2.55-2.44 (m, 2H), 2.38-2.21 (m, 2H), 2.15-1.96 (m, 5H), 2.03 (s, 3H), 1.96-1.79 (m, 7H), 1.76-1.67 (m, 3H), 1.67-1.50 (m, 7H), 1.33-1.19 (m, 12H), 1.08 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.3, 172.2, 170.7, 166.9, 159.4, 151.5, 141.8, 138.0,

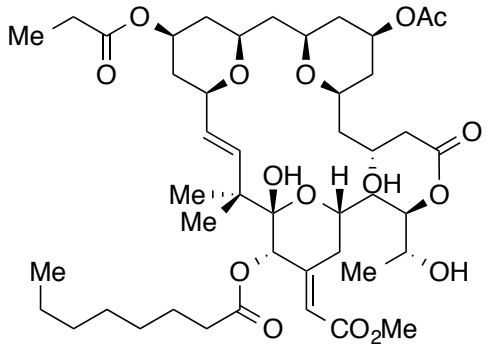
130.7, 129.7, 128.0, 127.9, 125.2, 119.5, 113.9, 103.3, 93.7, 78.9, 74.7, 73.6, 73.5, 73.4, 73.2, 71.9, 70.8, 70.3, 69.8, 68.3, 67.2, 55.5, 52.7, 51.4, 45.1, 43.6, 42.9, 42.3, 41.5, 41.2, 37.6, 37.5, 34.8, 34.7, 31.8, 31.0, 29.9, 29.2, 29.0, 26.5, 24.9, 22.8, 21.5, 20.1, 15.3, 14.3; 125 MHz DEPT NMR ($CDCl_3$) CH_3 δ 55.5, 52.7, 51.3, 26.5, 21.5, 20.1, 15.3, 14.3; CH_2 δ 93.7, 71.9, 69.8, 43.6, 42.9, 42.3, 41.5, 41.2, 37.6, 37.5, 34.8, 34.7, 31.8, 31.0, 29.2, 29.1, 24.9, 22.8; CH δ 141.8, 129.7, 128.6, 128.0, 127.9, 125.2, 119.5, 113.9, 78.9, 74.7, 73.6, 73.5, 73.4, 73.2, 70.8, 70.3, 68.3, 67.2; IR (neat): 3530, 2325, 2239, 1740, 1644, 1523, 1486, 1175, 1039, 886, 712 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{60}H_{84}O_{16}Na$ ($M + Na^+$) 1087.5601. Found: 1083.5619.



Preparation of (1*S*,3*R*,5*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10-dimethyl-19-oxo-5-(propionyloxy)-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 2.60. To a solution of the alcohol **2.59** (8 mg, 0.007 mmol, 1 equiv) in CH_2Cl_2 (750 μL, 0.01 M) was added pyridine (30 μL, 0.375 mmol, 50 equiv) followed by DMAP (9 mg, 0.075 mmol, 10 equiv) at rt. To this solution was added octanoic anhydride (28.5 μL, 0.225 mmol, 30 equiv) via syringe. The reaction mixture was stirred for 2 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The phases separated and

the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished using flash chromatography with a 0.5 × 10 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product (5-9) were combined and concentrated under reduced pressure to provide the product **2.60** (7.7 mg, 92%) as colorless oil: R_f = 0.50 (50% EtOAc/hexanes); $[\alpha]^{20}_D$ = +29.0 (*c* = 1.20, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.40-7.28 (m, 5H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.22 (d, *J* = 15.6 Hz, 1H), 5.93 (d, *J* = 1.6 Hz, 1H), 5.56 (ddd, *J* = 11.9, 6.1, 2.4 Hz, 1H), 5.29 (dd, *J* = 15.6, 8.5 Hz, 1H), 5.18 (s, 1H), 5.03 (ddd, *J* = 15.9, 11.0, 4.6 Hz, 1H), 4.86-4.77 (m, 1H), 4.83 (ABq, *J* = 7.1 Hz, Δν = 12.5 Hz, 2H), 4.65 (ABq, *J* = 11.9 Hz, Δν = 16.1 Hz, 2H), 4.48 (s, 2H), 4.21-4.15 (ddd, *J* = 13.2, 6.6, 3.7 Hz, 1H), 4.13 (m, 2 H), 4.03 (m, 1H), 3.95 (m, 1H), 3.75 (s, 3H), 3.72-3.65 (m, 2H), 3.61-3.53 (m, 1H), 3.69 (s, 3H), 3.60-3.53 (m, 1H), 3.52-3.44 (m, 4H), 3.16 (m, 1H), 3.06 (s, 3H), 2.55-2.44 (m, 2H), 2.34-2.23 (m, 2H), 2.15-1.96 (m, 5H), 2.03 (s, 3H), 1.96-1.79 (m, 7H), 1.76-1.67 (m, 3H), 1.67-1.50 (m, 7H), 1.33-1.19 (m, 12H), 1.08 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 174.0, 172.2, 172.2, 170.6, 166.9, 159.4, 151.5, 142.3, 138.1, 130.8, 129.7, 128.6, 128.0, 127.8, 124.9, 119.4, 113.9, 103.3, 93.8, 78.7, 75.1, 73.7, 73.5, 73.3, 73.3, 73.2, 71.9, 70.8, 70.4, 70.3, 69.8, 67.3, 66.0, 60.6, 55.5, 52.8, 51.3, 45.1, 43.7, 43.0, 41.2, 38.5, 37.6, 37.5, 34.8, 34.7, 31.8, 31.0, 29.9, 29.2, 29.0, 28.1, 26.4, 24.8, 22.7, 21.4, 21.2, 20.1, 15.5, 15.2, 14.4, 14.2, 9.4; 125 MHz DEPT NMR ($CDCl_3$) CH_3 δ 55.2, 52.5, 51.1, 26.1, 21.2, 19.8, 15.2, 14.2, 14.0; CH_2 δ 93.6, 71.7, 69.6, 65.8, 60.3, 43.4, 42.8, 40.9, 38.3, 37.4, 37.3, 34.6, 34.5, 31.6, 30.8, 29.7, 28.9, 28.8, 27.8, 24.6, 22.5; CH δ 142.0, 129.5, 128.4, 127.8, 127.6, 124.6, 119.2, 113.7, 78.5, 74.9, 73.4,

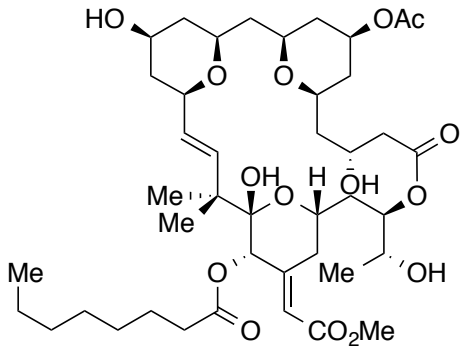
73.3, 73.1, 73.0, 72.9, 70.5, 70.1, 70.0, 67.0; IR (neat): 3430, 2225, 2139, 1746, 1654, 1519, 1485, 1175, 1001, 909, 886, 712 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{62}H_{88}O_{18}Na$ ($M + Na^+$) 1143.5863. Found: 1143.5875.



Preparation of (1*S*,3*R*,5*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-11,21-dihydroxy-17-((*R*)-1-hydroxyethyl)-13-(2-methoxy-2-oxoethylidene)-10,10-dimethyl-19-oxo-5-(propionyloxy)-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 2.9 (Merle 34). To a solution of the alcohol **2.60** (3.5 mg, 0.003 mmol, 1 equiv) in CH_2Cl_2 (624 μL, 0.005 M) was added *t*BuOH (6 μL) followed by pH 7 buffer (62 μL) at 0 ^oC. To this solution was added DDQ (3 mg, 0.012 mmol, 4 equiv). The reaction mixture was stirred for 1.5 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was passed through a plug of 0.25 × 8 cm silica gel column, eluting with 30% EtOAc/hexanes, collecting 1 mL fractions. The fractions containing product (5-9) were combined and concentrated under reduced pressure and directly taken for the next step.

To the crude product from the previous step (assumed to be 0.003 mmol) in a 5 mL vial was added $LiBF_4$ (540 μL, 0.25 M in 25:1 CH_3CN/H_2O, 0.135 mmol, 45 equiv) and heated to 80 ^oC for 10 h, after which the reaction mixture was cooled to rt. The solvent was evaporated under N_2 and then directly loaded to a 0.25 × 6 cm silica gel column. Purification was done by eluting with 5% MeOH/30% EtOAc/65% hexanes and collecting in 1 mL fractions. The fractions containing product (20-23) were combined and evaporated under reduced pressure to provide Merle 34 **2.9** (1.7 mg, 65% over 2 steps) as white foam: R_f = 0.1 (50% EtOAc/hexanes); $[\alpha]^{20}_D$ = +25.6 (*c* = 0.100, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.98 (d, *J* = 1.7 Hz, 1H), 5.80 (d, *J* = 15.8 Hz, 1H), 5.29 (dd, *J* = 15.8, 8.4 1H), 5.24-5.18 (m, 1H), 5.19 (s, 1H), 5.14 (s, 1H), 4.98 (ddd, *J* = 15.9, 11, 4.8 Hz, 1H), 4.84 (m, 1H), 4.39 (d, *J* = 12.1 Hz, 1H), 4.21 (m, 2H), 4.05 (m, 1H), 3.89-3.79 (m, 2H), 3.72-3.58 (m, 2H), 3.69 (s, 3H), 3.65-3.56 (m, 2H), 3.52 (m, 2H), 3.36 (m, 1H), 3.26 (m, 1H), 2.53 (dd, *J* = 12.3, 2.2 Hz, 1H), 2.48-2.40 (m, 1H), 2.35-2.24 (m, 2H), 2.11-1.98 (m, 4H), 2.04 (s, 3H), 1.96-1.78 (m, 8H), 1.68-1.48 (m, 4H), 1.36-1.18 (m, 10H), 1.13 (m, 3H), 0.99 (s, 3H), 0.89 (m, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 173.9, 172.3, 172.1, 170.6, 167.2, 152.0, 139.5, 136.0, 129.1, 125.7, 119.9, 99.1, 74.9, 74.4, 73.9, 73.6, 70.4, 69.7, 69.6, 68.7, 64.8, 51.2, 45.0, 42.9, 42.7, 40.0, 38.9, 38.2, 37.4, 36.1, 34.9, 34.5, 32.5, 31.9, 31.5, 30.6, 29.9, 29.5, 29.3, 29.0, 28.0, 27.5, 26.6, 24.9, 23.6, 22.8, 21.4, 20.0, 19.9, 14.3; 125 MHz, ^{13}C DEPT ($CDCl_3$) CH_3: δ 50.9, 30.3, 24.7, 21.1, 19.8, 19.7, 14.0; CH_2: δ 42.7, 42.5, 39.7, 38.7, 37.9, 37.2, 35.8, 34.6, 31.6, 31.2, 29.7, 28.9, 28.8, 27.8, 24.7, 22.5; CH δ 139.2, 128.9, 125.4, 119.6, 77.2, 77.1, 76.6, 74.7, 74.1, 73.6, 73.4, 70.2, 69.5, 69.3, 68.5, 64.5; IR (neat): 3395, 2924, 2852, 1746, 1655, 1426, 1275,

1110, 827, 660 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{45}H_{70}O_{16}Na$ (M + Na^+) 889.4556. Found 889.4569.



Preparation of (1*S*,3*S*,5*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*R*,25*S*)-25-acetoxy-5,11,21-trihydroxy-17-((*R*)-1-hydroxyethyl)-13-(2-methoxy-2-oxoethylidene)-10,10-dimethyl-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.$1^{3,7}$.$1^{11,15}$]nonacos-8-en-12-yl octanoate 2.10 (Merle 38). To a solution of the alcohol **2.59** (3.1 mg, 0.003 mmol, 1 equiv) in CH_2Cl_2 (582 μL, 0.005 M) was added *t*BuOH (5.8 μL) followed by pH 7 buffer (58 μL) at 0 ^oC. To this solution was added DDQ (2.6 mg, 0.012 mmol, 4 equiv). The reaction mixture was stirred for 1.5 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The phases separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was passed through a plug of 0.25 × 8 cm silica gel column, eluting with 50% EtOAc/hexanes, collecting 1 mL fractions. The fractions containing product (15-19) were combined and concentrated under reduced pressure and directly taken for the next step.

To the crude product from the previous step (assumed to be 0.003 mmol) in a 5 mL vial was added $LiBF_4$ (522 μL, 0.25 M in 25:1 CH_3CN/H_2O, 0.130 mmol, 45 equiv)

and heated to 80 ^{o}C for 10 h, after which the reaction mixture was cooled to rt. The solvent was evaporated under N_2 and then directly loaded to a 0.25 × 6 cm silica gel column. Purification was done by eluting with 5% MeOH/15% EtOAc/80% hexanes and collecting in 1 mL fractions. The fractions containing product (24-40) were combined and evaporated under reduced pressure to provide Merle 38 **2.10** (1.2 mg, 70% over 2 steps) as white foam: R_f = 0.46 (10% MeOH/30% EtOAc/60% hexanes); $[\alpha]^{20}_{D}$ = +18.2 (*c* = 0.120, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 5.98 (d, *J* = 1.7 Hz, 1H), 5.78 (d, *J* = 15.8 Hz, 1H), 5.30 (dd, *J* = 15.8, 8.4 1H), 5.24-5.18 (m, 1H), 5.22 (s, 1H), 5.14 (s, 1H), 4.84 (ddd, *J* = 15.9, 11, 4.8 Hz, 1H), 4.42 (d, *J* = 12.1 Hz, 1H), 4.21 (m, 2H), 4.05 (m, 3H), 3.93-3.78 (m, 3H), 3.73-3.66 (m, 1H), 3.69 (s, 3H), 3.65-3.56 (m, 2H), 3.51 (m, 2H), 2.53 (dd, *J* = 12.3, 2.2 Hz, 1H), 2.49-2.40 (m, 1H), 2.31 (ddd, *J* = 11.4, 7.7, 4.0 Hz, 2H), 2.11-1.98 (m, 4H), 2.04 (s, 3H), 1.96-1.78 (m, 8H), 1.68-1.48 (m, 4H), 1.36-1.18 (m, 10H), 1.13 (s, 3H), 1.00 (s, 3H), 0.88 (m, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.4, 170.7, 167.2, 152.0, 139.1, 129.4, 119.9, 99.1, 75.2, 74.3, 74.0, 73.6, 70.5, 69.6, 68.7, 67.6, 64.7, 51.3, 45.0, 42.9, 42.8, 42.6, 42.2, 39.9, 37.4, 36.0, 34.9, 31.9, 31.5, 29.9, 29.2, 29.1, 24.9, 24.9, 22.8, 21.4, 20.0, 19.9, 14.3; 125 MHz, ^{13}C DEPT ($CDCl_3$) CH_3: δ 51.0, 24.7, 21.2, 19.8, 19.7, 14.0; CH_2: δ 42.7, 42.6, 42.4, 41.9, 39.8, 37.2, 37.1, 35.8, 34.6, 31.6, 31.2, 29.7, 29.0, 28.9, 24.7, 22.5; CH δ 138.9, 129.2, 119.7, 77.3, 77.2, 76.7, 74.9, 74.1, 73.8, 73.4, 70.3, 69.4, 68.5, 67.4, 64.5; IR (neat): 3455, 2924, 2852, 1736, 1655, 1426, 1245, 1110, 827 cm^{-1}; HRMS (ESI/TOF) calcd for $C_{42}H_{66}O_{15}Na$ (M + Na^+) 833.4294. Found 833.4305.

References

1. (a) Hale, K. J.; Manaviazar, S., *Chem.-Asian J.* **2010**, *5*, 704-754; (b) Blumberg, P. M.; Pettit, G. R., In *New Leads and Targets in Drug Research*, Krosgaard-Larsen, P.; Christensen, C. B.; Kodof, H., Eds. Munksgaard: Copenhagen: 1992; p 273.

2. Berkow, R. L.; Kraft, A. S., *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116.

3. (a) Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4084-4088; (b) Kedei, N.; Lubart, E. S.; Lewin, N. E.; Telek, A.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Keck, G. E.; Kolusheva, S.; Jelinek, R.; Blumberg, P. M., *ChemBioChem* **2011**, *12*, 1242-1251; (c) Kedei, N.; Telek, A.; Czap, A.; Lubart, E. S.; Czifra, G.; Yang, D.; Chen, J.; Morrison, T.; Goldsmith, P. K.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Li, W.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2011**, *81*, 1296-1308; (d) Kedei, N.; Telek, A.; Michalowski, A. M.; Kraft, M. B.; Li, W.; Poudel, Y. B.; Rudra, A.; Petersen, M. E.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2013**, *85*, 313-324; (e) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *J. Am. Chem. Soc.* **2008**, *130*, 6660-6661; (f) Keck, G. E.; Li, W.; Kraft, M. B.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 2277-2280; (g) Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Peach, M. L.; Blumberg, P. M., *Angew. Chem., Int. Ed.* **2010**, *49*, 4580-4584; (h) Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 593-596.

4. (a) Keck, G. E.; Covel, J. A.; Schiff, T.; Yu, T., *Org. Lett.* **2002**, *4*, 1189-1192; (b) Yu, C.-M.; Lee, J.-Y.; So, B.; Hong, J., *Angew. Chem., Int. Ed.* **2002**, *41*, 161-163.

5. (a) Ng, S. B.; Guy, G. R., *Cell Signal* **1992**, *4*, 405-416; (b) Asiedu, C.; Biggs, J.; Lilly, M.; Kraft, A. S., *Cancer Res.* **1995**, *55*, 3716-3720; (c) Vrana, J. A.; Saunders, A. M.; Srikumar, P. C.; Grant, S., *Differentiation* **1998**, *63*, 33-42.

6. (a) Hommel, U.; Zurini, M.; Luyten, M., *Nat. Struct. Biol.* **1994**, *1*, 383-387; (b) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H., *Cell* **1995**, *81*, 917-924; (c) Xu, R. X.; Pawelczyk, T.; Xia, T. H.; Brown, S. C., *Biochemistry* **1997**, *36*, 10709-10717.

7. Bogi, K.; Lorenzo, P. S.; Szallasi, A.; Acs, P.; Wagner, G. S.; Blumberg, P. M., *Cancer Res.* **1998**, *58*, 1423-1428.

8. Peach, M., National Cancer Institute: 2012.

9. (a) Mancuso, A. J.; Huang, S.-L.; Swern, D., *J. Org. Chem.* **1978**, *43*, 2480-2482; (b) Mancuso, A. J.; Swern, D., *Synthesis* **1981**, 165-185; (c) Omura, K.; Swern, D., *Tetrahedron* **1978**, *34*, 1651-1660.

10. (a) Keck, G. E.; Geraci, L. S., *Tetrahedron Lett.* **1993**, *34*, 7827-7828; (b) Keck, G. E.; Krishnamurthy, D., *J. Am. Chem. Soc.* **1995**, *117*, 2363-2364; (c) Keck, G. E.; Krishnamurthy, D.; Grier, M. C., *J. Org. Chem.* **1993**, *58*, 6543-6544; (d) Keck, G. E.; Krishnamurthy, D.; Roush, W. R.; Reilly, M. L., *Org. Synth.* **1998**, *75*, 12-18.

11. (a) Keck, G. E.; Castellino, S.; Wiley, M. R., *J. Org. Chem.* **1986**, *51*, 5478-5480; (b) Keck, G. E.; Castellino, S., *J. Am. Chem. Soc.* **1986**, *108*, 3847-3849.

12. (a) Blaise, E. E., *Compt. Rend.* **1901**, *132*, 478; (b) Rinehart, K. L. J., *Organic Syntheses* **1955**, *35*, 15.

13. (a) Miyashita, A.; Yasuda, A.; Takaya, H.; Toriumi, K.; Ito, T.; Souchi, T.; Noyori, R., *J. Am. Chem. Soc.* **1980**, *102*, 7932-7934; (b) Noyori, R.; Ohta, M.; Hsiao, Y.; Kitamura, M.; Ohta, T.; Takaya, H., *J. Am. Chem. Soc.* **1986**, *108*, 7117-7119.

14. (a) Cherest, M.; Felkin, H., *Tetrahedron Lett.* **1968**, 2205-2208; (b) Wu, Y.-D.; Tucker, J. A.; Houk, K. N., *J. Am. Chem. Soc.* **1991**, *113*, 5018-5027; (c) Franck, R. W., Stereoelectronic Effects in Six-Membered Rings. In *Conformational Analysis and Stereochemistry of Six membered Rings*, Juaristi, E., Ed. VCH Publishers, Inc.: New York, 1995; pp 157-198.

15. Bunnelle, W. H.; Narayanan, B. A., *Organic Syntheses* **1993**, *8*, 602-605.

16. (a) Keck, G. E.; Truong, A. P., *Org. Lett.* **2005**, *7*, 2149-2152; (b) Truong, A. P. Dissertation Thesis, University of Utah, 2005; (c) Kraft, M. B. Dissertation Thesis, University of Utah, 2011.

17. (a) Parikh, J. R.; Doering, W. V. E., *J. Am. Chem. Soc.* **1967**, *89*, 5505-5507; (b) Lindgren, B. O.; Nilsson, T., *Acta Chem. Scand.* **1973**, *27*, 888-890; (c) Kraus, G. A.; Roth, B., *J. Org. Chem.* **1980**, *45*, 4825-4830; (d) Kraus, G. A.; Taschner, M. J., *J. Org. Chem.* **1980**, *45*, 1175-1176; (e) Bal, B. S.; Childers, W. E.; Pinnick, H. W., *Tetrahedron* **1981**, *37*, 2091-2096.

18. Lipshutz, B. H.; Harvey, D. F., *Synth. Commun.* **1982**, *14*, 267-277.

19. Armarego, W. L. H.; Perrin, D. D., *Purification of Laboratory Chemicals*. 4 ed.; Butterworth-Heinemann: Oxford: 1997.

20. Watson, S. C.; Eastham, J. F., *J. Organomet. Chem.* **1967**, *9*, 165-168.

21. Heumann, L. V. Dissertation Thesis, University of Utah, 2007.

22. Li, W. Dissertation Thesis, University of Utah, 2011.

CHAPTER 3

STUDIES TOWARDS THE SYNTHESIS OF C7, C9 DEOXY BRYOSTATIN ANALOGUE

Introduction

The bryostatins are an important class of antitumor natural products that bind to the C1 domain of PKC isozymes with very high binding affinity.[1] Bryostatin 1 is the most thoroughly studied member of this family and has been subjected to numerous phase I and II clinical trials for cancer chemotherapy.[2] Additionally, it has been shown to reverse multidrug resistance in cancer cells, synergize with other anticancer agents like Taxol®, stimulate the immune system, improve learning and memory in animal models, and reduce the formation of β-amyloid plaques. It is also going through a clinical trial for Alzheimer's disease. Recently, it has also been shown to activate latent HIV in lymphocytes.[3]

The unique biological profile of bryostatin 1 has been attributed to the fact that bryostatin 1 binds to PKC with exceptionally high affinity.[3e] Our group has endeavored to find out the exact structural features of bryostatin 1 for its unique biological profile via rational design of synthetic analogues. With the syntheses of Merle 23, 27, 28, 30, 32, 33, 34, and 38, we developed considerable insight on the structural features of bryostatin behind its responses towards various biological endpoints.[4] The biological results with

Merle 23 refuted the concept that the northern hemisphere of bryostatin 1 is a mere spacer domain and holds together the key pharmacophoric elements C1 carbonyl oxygen, C19 hydroxy oxygen, and C26 hydroxy oxygen. Merle 23 lacked all the functional groups at the northern hemisphere of bryostatin 1 and Merle 28 reintroduced all the functional groups except the C30 carbomethoxy group. Merle 28 showed close resemblance to bryostatin while Merle 23 was similar to PMA in proliferation and attachment assays with U937 cells. With the biological evaluation of Merle 27, 30, and 32, we came to the conclusion that C7 acetate, C9 hydroxy; C8 gem-dimethyl groups are not solely responsible towards bryostatin's biology. However, it became clear that combinations of these groups are necessary for the unique biology.

To explore the idea we proposed that incorporation of two polar functional groups in the bryopyran, making it similar in hydrophilicity to that of bryostatin 1, should be capable of retaining bryostatin biology. This polarity-based hypothesis has been explained in Chapter 2 and will not be further discussed here. However, the conclusion of the biological studies with Merle 34 and 38 has shed some light on the crucial fact that the C13-C30 olefinic functionality with the carbomethoxy group appears to have some role when in combination with the C7 acetate. The specific role is not clear but the relatively lower binding affinities with PKC for Merle 27 (K_i = 3.00 ± 0.6 nM) compared to Merle 30 (K_i = 0.38 nM) and 33 (K_i = 0.68 nM) was the first indication in that direction. This specific effect is likely to get overshadowed by the presence of other functional groups such as in Merle 28 (K_i = 0.52 ± 0.06 nM). The presence of an ester moiety or a free hydroxy group at C13 dramatically lowered the binding affinity from that of Merle 33 to those of Merle 34 (K_i = 16.3 nM) and 38 (K_i = 13.21 nM). This

forced us to further investigate the role of the C13-C30 olefin with the carbomethoxy group. In line with this strategy, we proposed the synthesis of an analogue, which will delete both the C7 and C9 hydroxy groups and retain only the C13-C30 carbomethoxy enoate functionality. This will enable us to directly compare this analogue (Merle 40) with Merle 30 and 32 and will lead to a better understanding of the individual role of this functionality.

1st-generation retrosynthetic analysis of Merle 40

With the synthesis of Merle 30 and 32, we developed a route where we could selectively hydrolyze the C7 acetate in the presence of the macrolactone ester linkage at C1. We realized that the intermediate **3.2** (Figure 3.1) in the synthetic route to Merle 32 could also be used for the synthesis of Merle 40. Barton-McCombie deoxygenation could

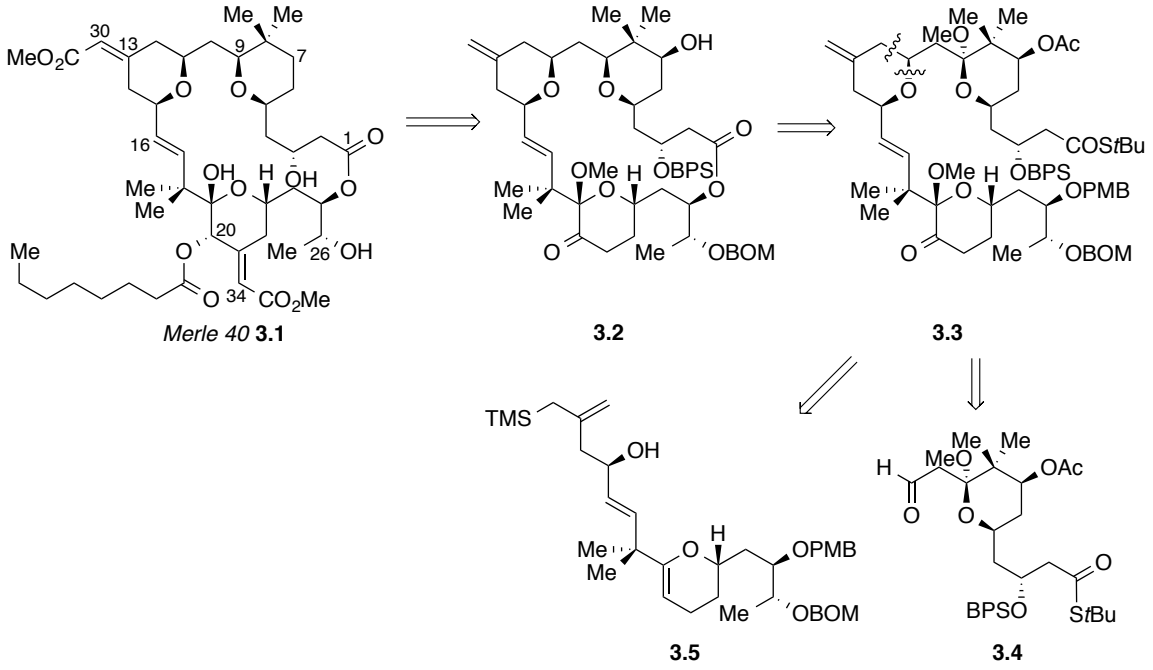


Figure 3.1. 1st-generation retrosynthetic analysis of Merle 40

be utilized to delete the C7 hydroxyl of **3.2**.[5] Merle 32 can be obtained from the known precursor intermediate **3.3**. The advanced intermediate **3.3** was synthesized earlier from the A-ring aldehyde **3.4** and the β-hydroxyallyl silane **3.5**. However, before pursuing this route any further, we wanted to study the methods to delete the C7 oxygenated functionality in model substrates. The methods developed from this strategy could then be utilized on the alcohol **3.2** to delete the C7 hydroxyl.

Model studies for the Barton-McCombie deoxygenation

The A-ring alcohol **3.6**, an intermediate en route Merle 32, was protected as the TBS ether to give **3.7**. Attempts were then made to hydrolyze the C7 acetate in the presence of the thioester to provide the free alcohol (Figure 3.2). Various attempts failed to hydrolyze the acetate selectively without affecting the thioester. Eventually, we settled

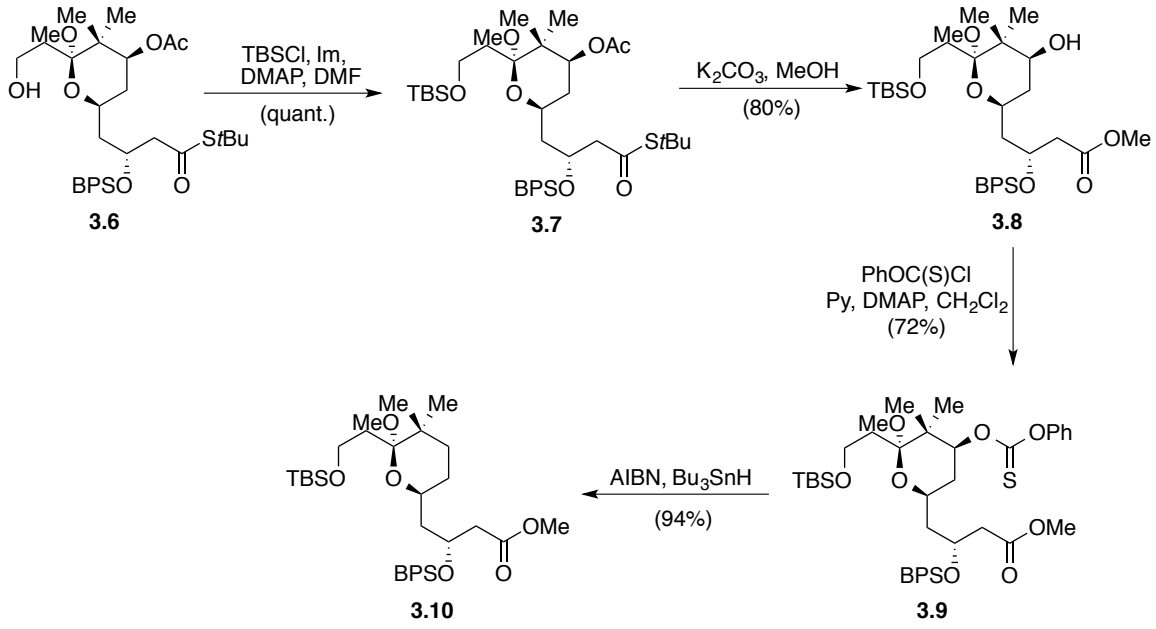


Figure 3.2. Model studies of Barton-McCombie deoxygenation

with a compromise in that the transesterification of the thioester occurred along with hydrolysis of the C7 acetate. K_2CO_3/MeOH conditions targeted both the acetate and the thioester and TLC analysis showed the formation of two new spots. The reaction was continued until the spots merged to a single spot after 2-3 h. The free hydroxy at C7 was then converted to the thionoester **3.9**. With the thionoester **3.9** in hand, we attempted the Barton-McCombie deoxygenation and to our delight, we observed the deoxygenation went smoothly to give the C7 deoxy compound **3.10**.

However, at this point of time, a 2nd-generation synthetic route for the total synthesis of bryostatin 1 was being explored where a more convergent coupling with a fully functionalized C-ring aldehyde and an A-ring β-hydroxyallyl silane was utilized. With the successful application of this 2nd-generation route, we decided to utilize a similar route with the fully functionalized C-ring aldehyde and thus avoid various side reactions that occurred with the late-stage functionalization of C-ring.

2nd-generation retrosynthesis of Merle 40

The 2nd-generation retrosynthetic analysis of Merle 40 followed the same strategy as for bryostatin 1, Merle 34, and 38 (Figure 3.3). A disconnection at the C1 ester bond keeping late-stage Yamaguchi macrolactonization in mind and the second disconnection at the B-ring pyran ring across C15 carbon led to the two equally complex synthetic intermediates β-hydroxyallyl silane **3.12** and the C-ring aldehyde **3.11**. The aldehyde **3.11** could arise from some protecting group manipulation of a previously known aldehyde with PMB ether at C25. The β-hydroxyallyl silane **3.12** was envisaged to form via Corey-Williams type allylation with the silane **3.13** onto the aldehyde **3.14**.[6] The aldehyde

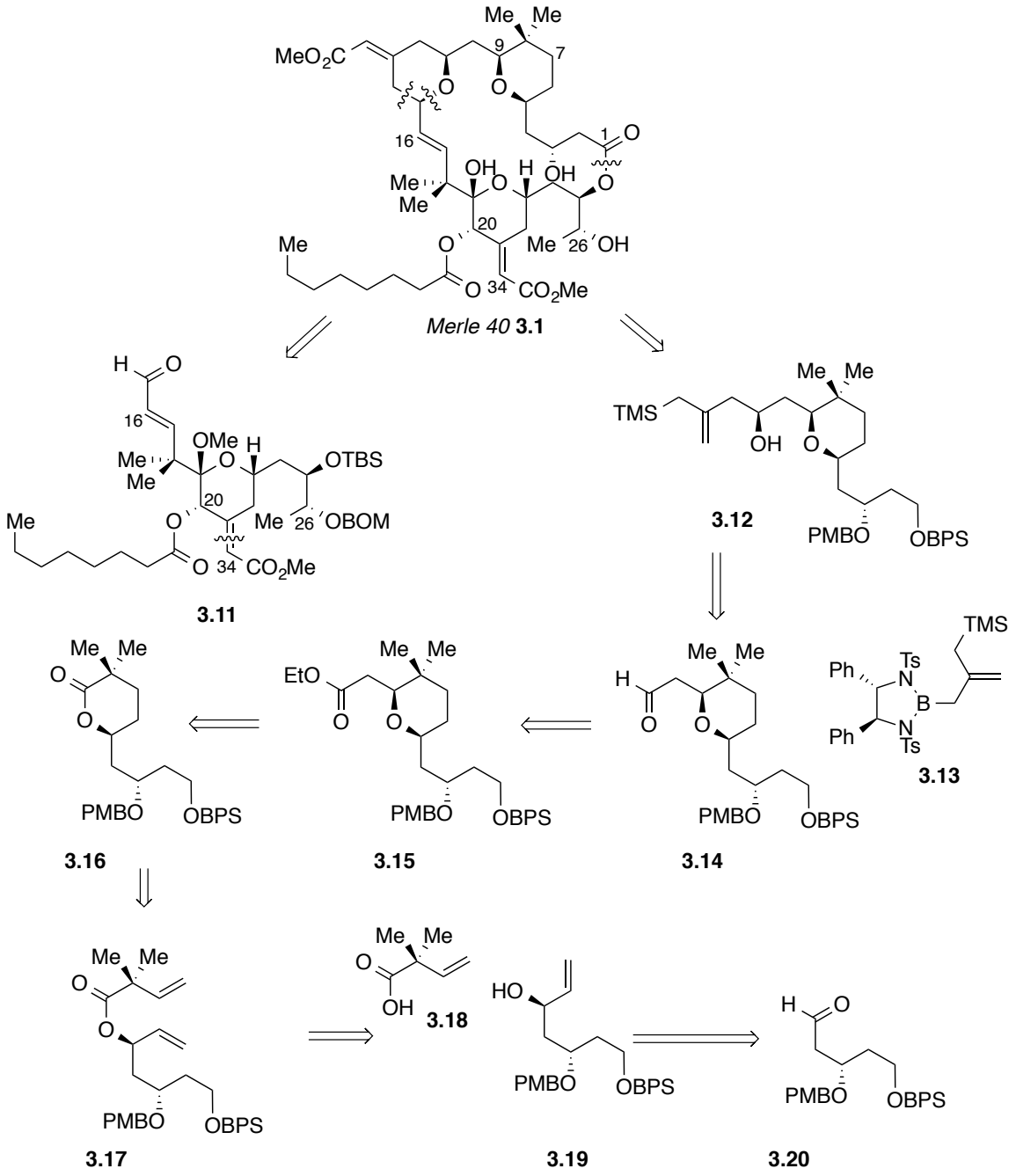


Figure 3.3. 2nd-generation retrosynthesis of Merle 40

3.14 could arise from the ethyl ester **3.15** via a DIBAL-H half reduction. The ester **3.15** was envisioned to arise from an addition of ethyl acetate onto the lactone **3.16** followed by reduction of the hemiketal at C9 of the adduct. The lactone **3.16** could arise from a ring-closing metathesis of the ester **3.17** followed by hydrogenation of the resulting alkene. Although there are very few examples of a ring-closing metathesis next to a gem-dimethyl group, we anticipated that free energy of the formation of six-membered ring from the tethered alkenes would drive the reaction.[7] The ester **3.17** could then arise from esterification of the alcohol **3.19** with the known 3,3-dimethyl-2-butenoic acid **3.18**. The known carboxylic acid **3.18** could be obtained from tiglic acid via a known procedure.[8] The alcohol **3.19** was disconnected at the C5 stereocenter, which could arise from a chelation-controlled stereoselective vinylation of the previously synthesized aldehyde **3.20**.

Synthesis of Merle 40

Synthesis of the β-hydroxyallyl silane **3.12**

The synthesis of the A-ring β-hydroxyallyl silane **3.12** began with the monoprotection of 1,3-propanediol **3.21** followed by Swern oxidation to provide the aldehyde **3.23**. Using catalytic asymmetric allylation (CAA) developed in our group with (*R*)-BITIP catalyst, we were able to convert the aldehyde to the homoallylic alcohol **3.24**. Protection of the alcohol as the PMB ether followed by ozonolysis of the terminal alkene provided the aldehyde **3.20**. With the aldehyde **3.20** in hand, we attempted various Lewis acid-mediated 1,3 chelation-controlled addition of a vinyl Grignard reagent. Strong Lewis acids such as $SnCl_4$, $TiCl_4$ mostly decomposed the aldehyde with only traces of the

desired product formed. Mild Lewis acids such as triflates of the lanthanide metals (Sm, Ln), on the other hand, did not provide any selectivity possibly due to a lack of strong co-ordination with the aldehyde. When we switched our Lewis acid to dimethyl aluminum chloride, we found it to realize diastereoselectivity (2:1), albeit low. At this point, we realized that commercially available vinyl magnesium bromide is in a co-ordinating solvent THF, which could potentially disrupt the chelation required for higher diastereoselectivity. Dr. Merritt Andrus from our group previously observed that removal of THF *in vacuo* and replacing the solvent with CH_2Cl_2 provided a 50-100-fold increase in diastereoselectivity in such reactions.[9] In our case, as the Lewis acid dimethyl aluminum chloride is in toluene, we decided to replace THF with toluene. To our delight, we observed an increase in diastereoselectivity to 10:1 under these conditions. However, attempts at further optimization with these conditions did not yield any increase in diastereoselectivity. We then focused on proving the stereochemistry of the C5 hydroxy. Under the chelation control, we anticipated 10:1 diastereoselectivity favoring the desired 1,3-*anti* relative configuration between C3 and C5. Protection of the free alcohol **3.26** as the acetate, removal of the PMB group, followed by revealing the C5 hydroxy group provided the diol, which was then converted to the acetonide **3.27**. To our utter surprise, NMR analysis of the acetonide using Rychnovsky's method revealed that the major diastereomer was of the 1,3-*syn* relative configurations between C3 and C5.[10] The minor diastereomer was the desired product and NMR analysis again supported that (Figure 3.4). The mechanism of this outcome is not understood at this point as a second chelation with sterically hindered BPS ether seemed too far-fetched to explain the stereochemistry. Luckily for us, the next step was to esterify the C5 hydroxy group and hence this

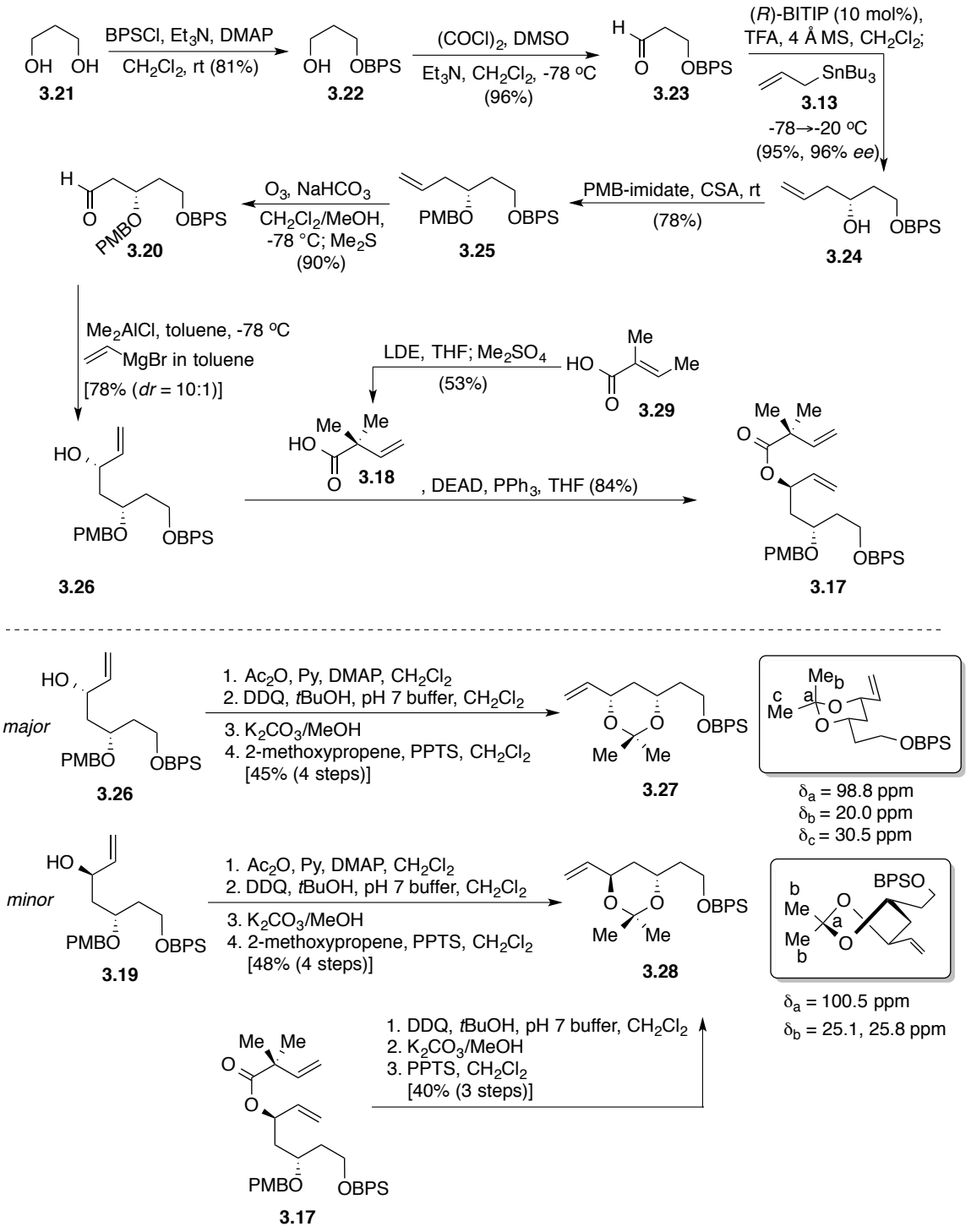


Figure 3.4. Synthesis of **3.17** and proof of C5 stereochemistry

outcome did not jeopardize our synthetic route as we decided to utilize Mitsunobu esterification with the carboxylic acid **3.18** to switch the C5 stereocenter to the desired 1,3-anti relative configuration with C3.[11] The 3,3-dimethyl-2-butenoic acid **3.18** was prepared in one step from commercially available tiglic acid in moderate yield via enolization of one of the protons on β-methyl group followed by capturing the enolate with an electrophilic methyl source (Me_2SO_4) at the α position. With **3.17** in hand, we wished to ensure that the stereocenter at C5 had indeed switched to the desired one. Following similar steps as before, we converted **3.17** to the acetonide **3.28** and NMR analysis proved the stereocenter had indeed switched to the desired one.

With the ester **3.17** in hand, we then attempted ring-closing metathesis reaction with Grubbs' 1st- and 2nd-generation catalysts. However, we did not detect any product formation even using a stoichiometric amount of catalyst under reflux conditions at 110 ^{o}C. We considered that the strong co-ordination of the catalyst with multiple oxygenated functionalities on the substrate could be the reason behind the complete inactivity of the catalysts. Literature precedence for the use of Ti(O*i*Pr)$_4$ under similar circumstances prompted us to reflux the substrate in CH_2Cl_2 in the presence of 5 equivalent of Ti(OiPr)$_4$ and then add the Grubbs' 2nd-generation catalyst **3.30**.[12] To our delight, the reaction went to completion cleanly after refluxing overnight at 45 ^{o}C (Figure 3.5). With the ring-closed alkene **3.31** in hand, we then attempted to hydrogenate the alkene without affecting the PMB ether. Various hydrogenating agents such as Pd/H_2 in charcoal, Pd(OAc)$_2$/H_2, tosyl hydrazine, and Ru(PPh$_3$)$_2$Cl$_2$/H_2 resulted in complex mixture of products including hydrogenation of C6-C7 bond and cleavage of PMB ether at C3. Finally, Adams' catalyst under atmospheric pressure of H_2 at 0 ^{o}C within 1 h gave the desired hydrogenation

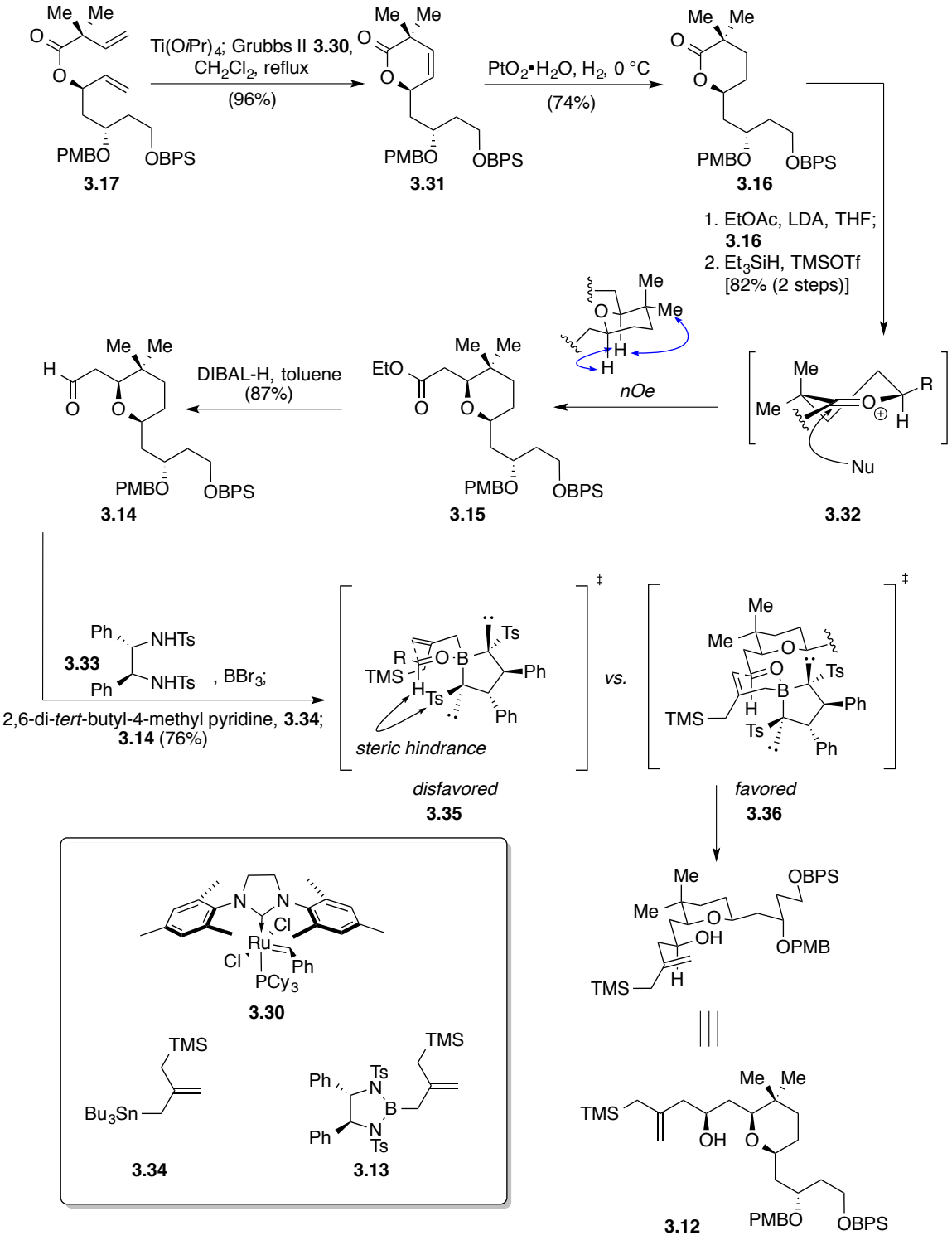


Figure 3.5. Synthesis of the β-hydroxyallyl silane **3.12**

product **3.16**. It proved necessary to monitor the reaction closely and remove the hydrogen atmosphere after about 1 h to prevent the cleavage of the PMB ether linkage. The next step was to add an enolate nucleophile onto the ester carbonyl at C9 to form the hemiketal. However, this apparently straightforward reaction proved challenging as the enolate of ethyl acetate could not be added onto the ester **3.16** under various conditions. We anticipated that the presence of any trace amount of Ru complex carried over from the previous metathesis reaction might be deleterious for the enolate to survive under the reaction condition. Therefore, our focus shifted towards getting rid of any trace amount of Ru from the substrate. Stirring the reaction mixture with charcoal overnight got rid of most of the Ru complexes. Although column chromatography on the crude reaction mixture was able to purify the product, it still retained a faint brownish color of Ru complexes. NMR analysis could not detect this trace amount of Ru complex. Fortunately, under oxidative work-up condition when a CH_2Cl_2 solution of the product was treated with H_2O_2, the brownish color disappeared after effervescence, indicating complete destruction of Ru complexes to Ru oxides.[13] After complete removal of any trace amount of Ru, the lactone **3.16** was reacted with the enolate of ethyl acetate and the reaction went smoothly to give the intermediate hemiketal. This hemiketal was subsequently subjected to reductive etherification condition with Et_3SiH and TMSOTf to produce the A-ring substituted pyran **3.15**. The reaction can be expected to proceed through a half-chair reactive intermediate **3.32** where the hydride source approaches the oxocarbenium ion from the bottom face to achieve relatively stable chair-like transition state and avoid a high energy twist boat type transition state in case of top face attack. The stereochemistry of C9 was proven by nOe experiments in NMR analysis. Interestingly, during nOe

experiments, we observed that the C9 hydrogen correlated with the protons of one of the C8 gem-dimethyl groups that were upfield. One would expect a nOe enhancement of the signal for the C8 equatorial methyl protons, which are usually downfield. A gradient homonuclear correlation spectroscopy (gCOSY) NMR experiment and a gradient heteronuclear multiple quantum coherence (gHMQC) NMR experiment revealed that the upfield protons were indeed those of the C8 equatorial methyl group. The original assignment of these protons by George Pettit was erroneous and later revised by Schaufelberger.[14]

DIBAL-H reduction of the ethyl ester **3.15** provided the half-reduction product aldehyde **3.14**. With this aldehyde in hand, we attempted Williams modified Corey allylation using the 2-substituted allyl silane **3.13**. In 1989, Corey reported an asymmetric allylation with tosylated (*S*,*S*)-1,2-diamino-1,2-diphenylethane (also known as stilbenediamine, stien) as the ligand, BBr_3 as the Lewis acid, and allyl stannane as the transmetalating agent.[6c, 6d] Williams modified this procedure with 2-substituted allyl stannane reagents.[6a, 6b, 6e] The ligand had to be moisture free as traces of water often decomposed the boron-ligand adduct. It was also necessary to remove all the residual volatile brominated compounds for a successful reaction. The presence of 2,6-di-*tert*-4-methyl pyridine was used to sequester any acidic protons from hydrogen bromide formed as the by-product. The active allyl borane reagent **3.13** was generated via transmetalation by the allyl stannane reagent **3.34**, which successfully converted the aldehyde **3.14** to the β-hydroxy allyl silane **3.12**. The stereo induction of the C11 of **3.12** can be explained by considering a favored transition state like **3.36** where the *Si* face of the aldehyde is attacked by the allyl borane reagent. The tosyl groups on the nitrogen atoms of the ligand

are oriented so as to decrease the steric repulsion with the phenyl groups. The absence of the steric hindrance between the aldehyde carbonyl proton and one of the tosyl group makes **3.36** favored over the other transition state **3.35** for *Re* face attack. The stereochemistry of the C11 alcohol was proved by Mosher ester analysis of the alcohol **3.12**.[15] Both (*R*) and (*S*) Mosher esters were prepared and compared for the $\Delta\delta_{S\text{-}R}$. The most stable conformations are indicated as **3.37** and **3.38** for (*R*) and (*S*) MTPA, respectively. For (*R*)-MTPA ester, the portion colored in green (Figure 3.6), denoted as R_2, gets shielded by the phenyl group whereas for (*S*)-MTPA ester, the orange colored portion of the molecule R_1 gets shielded by the phenyl group. Therefore, the $\Delta\delta_{S\text{-}R}$

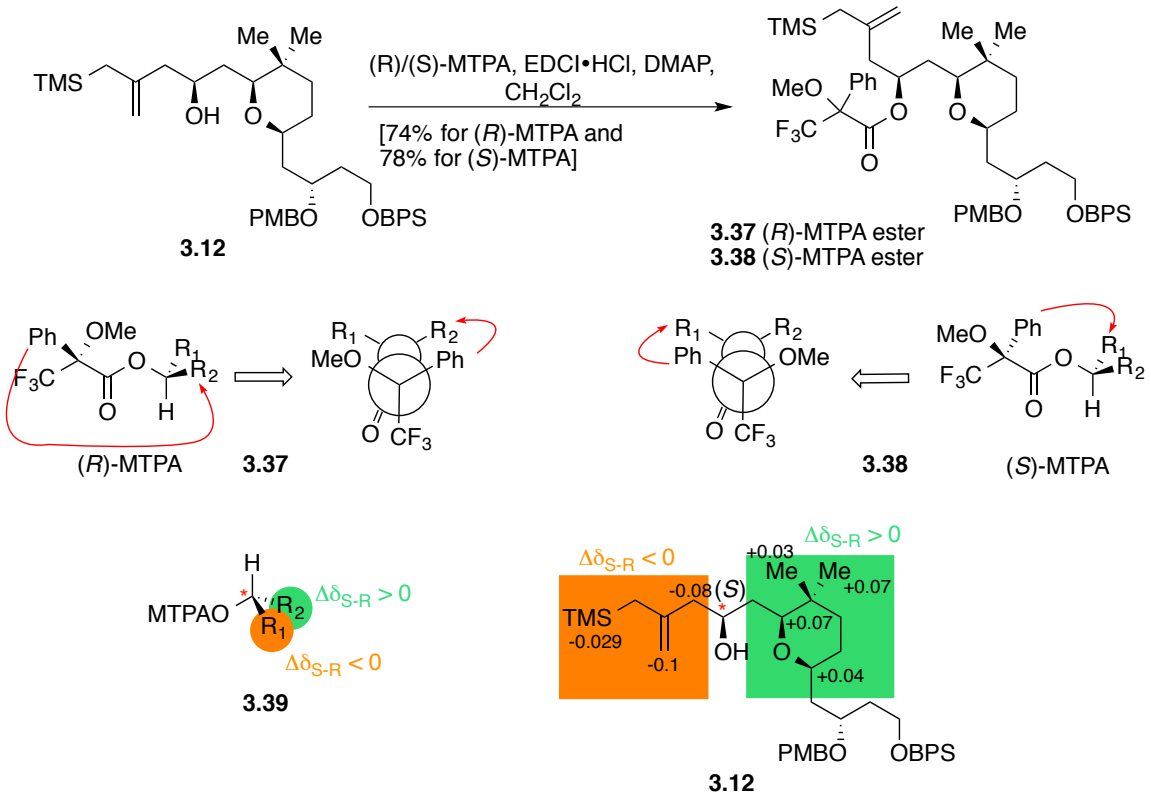


Figure 3.6. Mosher ester analysis for the stereochemistry at C11 of **3.12**

is positive for R_2, whereas it is negative for R_1. R_1 and R_2 were then placed according to the prescribed suggestion of Mosher. The analysis (Figure 3.6) indicated the absolute stereochemistry of C11 to be (*S*) as expected.

Completion of the synthesis of Merle 40

With the β-hydroxyallyl silane **3.12** in hand, we then attempted to couple with the fully functionalized C-ring **3.11** using the key reaction of pyran annulation. The standard conditions of pyran annulation, however, could not achieve the annulation and only trace amount of the desired product **3.40** was isolated (Figure 3.7). The major side products were the protodesilylation of the β-hydroxyallyl silane **3.12**, TMS protection of the free alcohol at C11, and elimination of the methoxy ketal at C19. The lack of reactivity of the aldehyde **3.11** towards the silane **3.12** was unexpected considering its success in the total syntheses of bryostatin 1 and 7 and hence is not fully understood. However, isolation of TMS protected silane prompted us to introduce a free hydroxyl group in the substrates. In the total syntheses of Merle 33, 34, and 38, it had been observed that free hydroxy

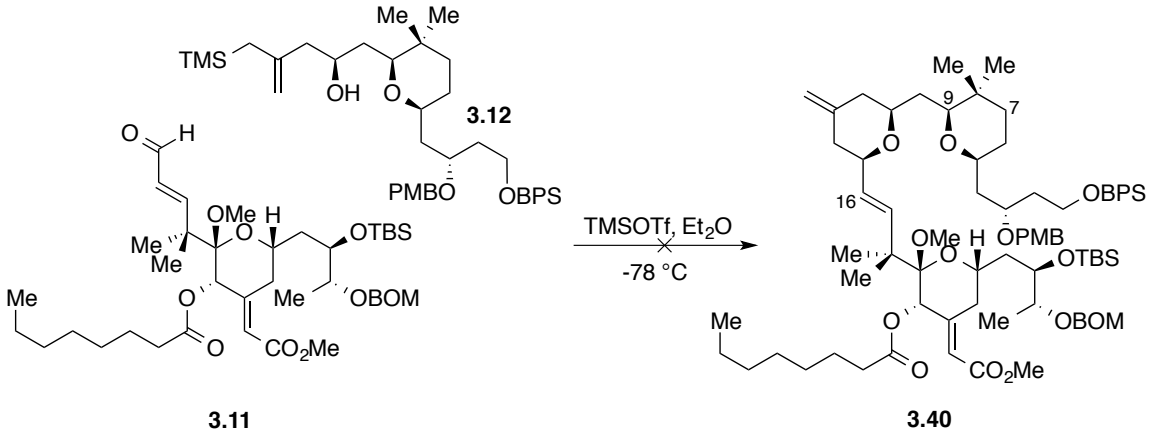


Figure 3.7. Failed attempt for pyran annulation

groups on the substrates are well tolerated in this reaction. Therefore, we decided to use a synthetic equivalent of the C-ring aldehyde **3.11** with a free hydroxyl at C25 (Figure 3.8).[16] Deprotection of the C25 TBS group of **3.11** gave the aldehyde **3.41**. To our delight, the pyran annulation of **3.41** with **3.12** went smoothly in about 2 h to provide **3.42**. Protection of the C25 free alcohol provided the compound **3.40**. Next, removal of the BPS group followed by Parikh-Doering oxidation and Lindgren oxidation provided the carboxylic acid at C1.[17] Removal of the TBS ether of C25 gave the seco-acid, which was then subjected to Yamaguchi macrolactonization to provide the macrolactone **3.44**.[18] Using a saturated solution of ozone at -78 ^{o}C, we were able to selectively cleave the olefin at C13-C30. All that is left is to convert the ketone to the carbomethoxy enoate **3.45** using Fuji's chiral phosphonate **3.47**[19] followed by removal of the PMB ether at C3 and global deprotection to provide the analogue Merle 40 (Figure 3.9).

Conclusion

Development of a synthetic strategy towards the analogue Merle 40 was accomplished. This synthetic route also demonstrated the scope of pyran annulation under different circumstances. Along with the total syntheses of Merle 33, 34, and 38, this synthetic route established the fact that pyran annulation can in certain circumstances go smoothly in presence of free hydroxyl groups. A new stereochemical outcome was observed during a chelation-controlled addition with vinyl Grignard reagent. A sterically demanding metathesis reaction was achieved in presence of various other co-ordinating groups. Additionally, Williams modified Corey allylation elegantly installed the C11 stereocenter. The A-ring β-hydroxyallylsilane was synthesized in just 12 steps. Another

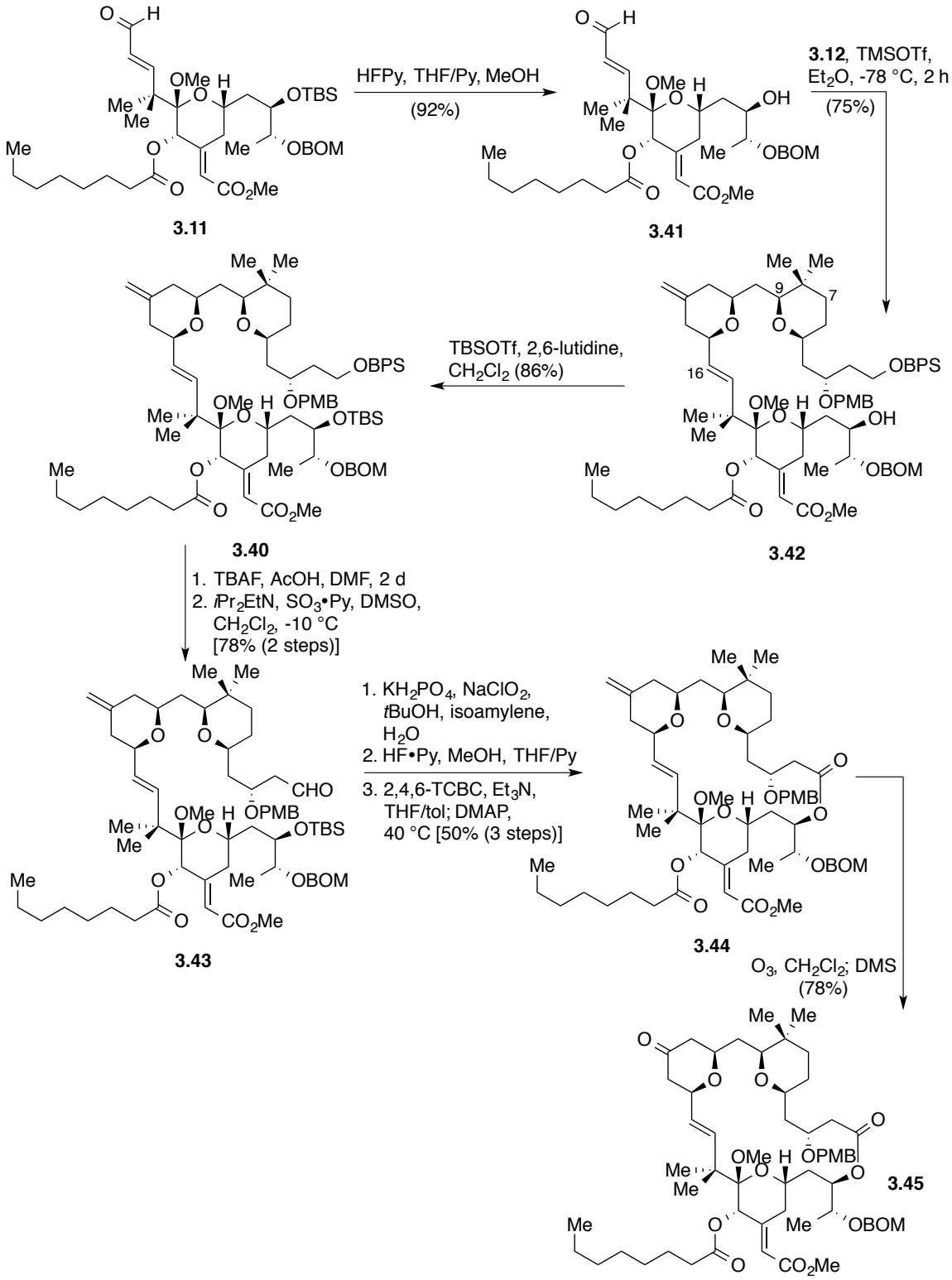


Figure 3.8. Synthesis of the ketone **3.45**

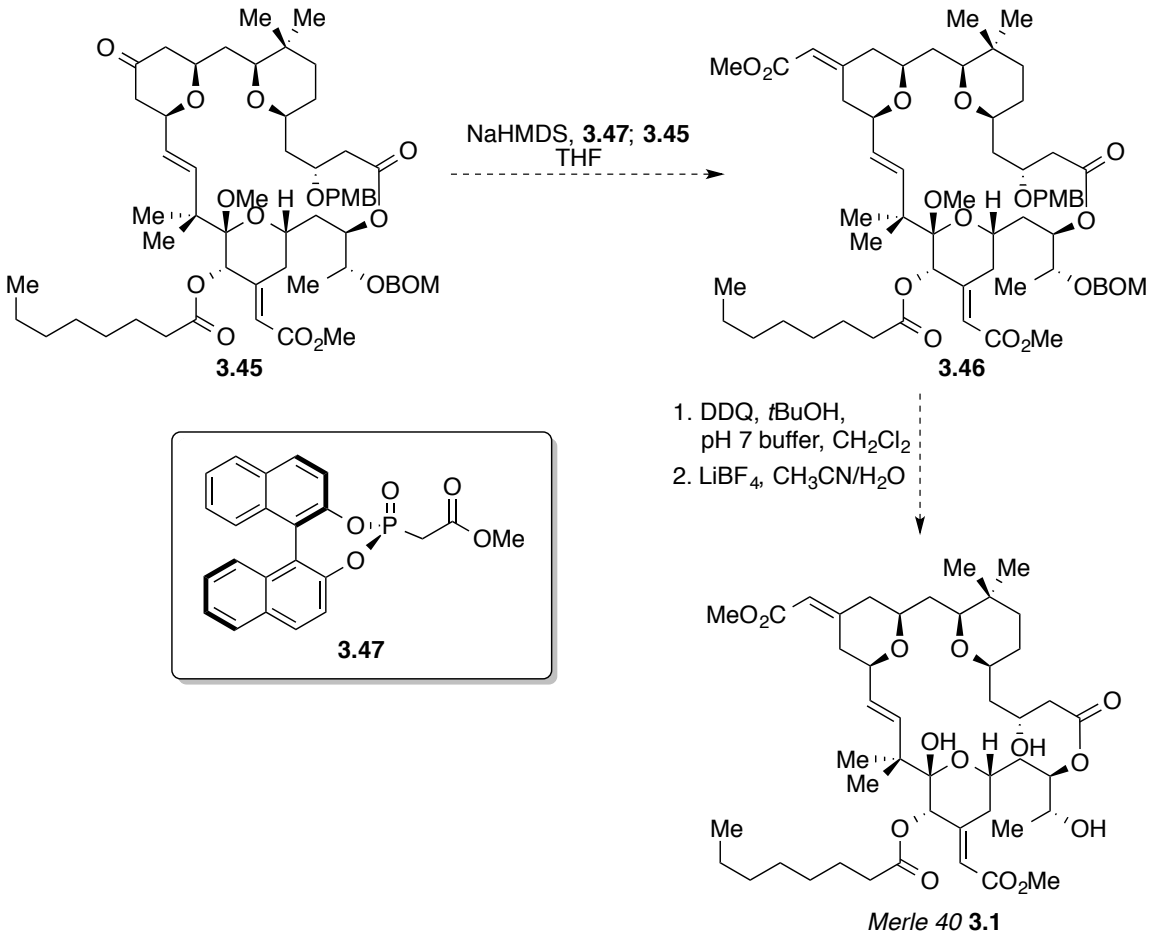


Figure 3.9. Completion of the synthesis of Merle 40

11 steps would accomplish the synthesis of Merle 40 taking the longest linear steps to just 33.

The biological studies with Merle 40 should reveal the role of the C13-C30 carbomethoxy enoate group compared to merle 30, 32. Additionally, these studies should also provide further insights into our current understanding of the interaction of the ligand, C1 domain, and the phospholipid.

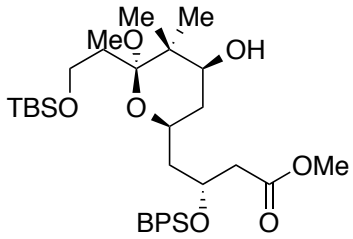
Experimental section

General experimental procedures, materials, and instrumentation

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals*.[20] Diisopropylamine, triethylamine, pyridine, Hünig's base, EtOAc, and CH_2Cl_2 were distilled from CaH_2 under an atmosphere of dry N_2. THF, Et_2O, and toluene were distilled from Na under an atmosphere of dry N_2. $Ti(O\textit{i}Pr)_4$ and $TiCl_4$ were distilled prior to use. A stock solution of $Ti(O\textit{i}Pr)_4$ (1.0 M in CH_2Cl_2) was prepared and used for the BITIP catalyst preparations. The titer of *n*-butyllithium was determined by the method of Eastham and Watson.[21] All other reagents were used without further purification. Yields were calculated for material judged homogeneous by thin layer chromatography and nuclear magnetic resonance (NMR) spectroscopy. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} plates eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid. Glassware for reactions was oven dried at 125 ^{o}C and cooled under a dry atmosphere prior to use. Liquid reagents and solvents were introduced by oven-dried syringes through septum-sealed flasks under a nitrogen atmosphere. Column flash chromatography was performed with Silicycle Grade 70 – 230 mesh, 60 – 200 μm, 60 Å silica gel, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Analtech Inc. Silica Gel GF 20 cm × 20 cm × 2000 μm plates or on Merck Kieselgel 60 F_{254} 20 cm × 20 cm × 250 μm plates. Nuclear magnetic resonance spectra were acquired on Varian VXR-500, Varian Inova-500 spectrometer 500 MHz for ^{1}H and 125 MHz for ^{13}C. Prior to use, $CDCl_3$ was filtered through a plug of Fischer Scientific 80 – 200 mesh Alumina

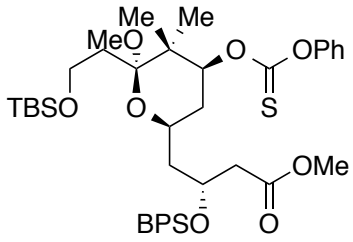
Adsorption stored at 110 ^{o}C. Chemical shifts for proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the signal of residual $CHCl_3$ at 7.27 ppm, or relative to the signal of residual C_6D_5H at 7.16 ppm. Chemical shifts for carbon nuclear magnetic resonance (^{13}C and DEPT) spectra are reported in parts per million relative to the signal of trimethylsilane at 0 ppm, relative to the center line of the $CDCl_3$ triplet at 77.23 ppm, or relative to the center line of the C_6D_6 triplet at 128.62 ppm. Chemical shifts of the unprotonated carbons ('C') for DEPT spectra were obtained by comparison with the ^{13}C NMR spectrum. The abbreviations s, bs, d, dd, ddd, dddd, t, td, tt, q, dq, dqd, ddq, ABq, quin, and m stand for the resonance multiplicity singlet, broad singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, doublet of quartet of doublets, doublet of doublet of quartets, AB quartet, quintet, and multiplet, respectively. IR spectra were obtained from a Perkin Elmer FT-IR Paragon 1000 PC spectrometer. Melting points were obtained using a Mel-Temp electrochemical melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin Elmer model 343 polarimeter (Na D line) using a microcell with 1 dm path length. Specific rotations ($[\alpha]^{20}_{D}$, Unit: $^{o}cm^2/g$) are based on the equation $\alpha = (100.\ \alpha)/(l.c)$ and are reported as unitless numbers where the concentration c is in g/100 mL and the path length l is in decimeters. Mass spectrometry was performed at the mass spectrometry facility of the Department of Chemistry at the University of Utah on a Finnigan MAT 95 double focusing high-resolution mass spectrometer. Compounds were named using ChemBioDraw 13.0.

Experimental procedures and analytical data



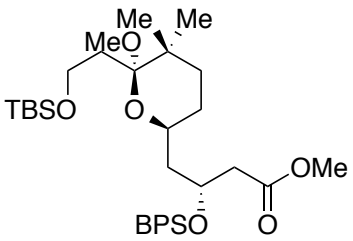
Preparation of methyl (*R*)-4-((2*S*,4*S*,6*S*)-6-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-4-hydroxy-6-methoxy-5,5-dimethyltetrahydro-2*H*-pyran-2-yl)-3-((*tert*-butyldiphenylsilyl)oxy)butanoate 3.8. To a stirring solution of the acetate **3.7** (103.8 mg, 0.134 mmol, 1.00 equiv) in MeOH (2.7 mL, 0.05 M) in a 10 mL round-bottom flask under an atmosphere of N_2 was added K_2CO_3 (92.7 mg, 0.671 mmol, 5.00 equiv) in one portion. The mixture was stirred for 6 h and then quenched by the addition of saturated aqueous NH_4Cl solution (5 mL). The phases were separated and the organic phase was extracted with 30% EtOAc/ hexanes (3 × 15 mL). The organic phases were combined, washed once with brine (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 1.5 × 15 cm silica gel column, eluting with 30% EtOAc/ hexanes (250 mL), collecting 5 mL fractions. The fractions containing product (13 - 17) were combined and concentrated under reduced pressure to give the alcohol **3.8** (72.2 mg, 80%) as colorless oil: R_f 0.26 (30% EtOAc/ hexanes); $[\alpha]^{D}_{20}$ = +25.5 (*c* = 0.88, $CHCl_3$); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.71 – 7.64 (m, 4H), 7.46 – 7.35 (m, 6H), 4.28 – 4.22 (m, 1H), 3.78 – 3.64 (m, 2H), 3.59 (s, 3H), 3.60 – 3.54 (m, 1H), 3.20 (ddd, *J* = 11.5, 7.1, 3.8 Hz, 1H), 2.93 (s, 3H), 2.67 (dd, *J* = 14.7, 4.2 Hz, 1H), 2.55 (dd, *J* = 14.8, 7.8 Hz, 1H), 1.94 – 1.69 (m, 3H), 1.52 (td, *J* = 14.0, 3.9 Hz, 1H), 1.26 (m, 1H), 1.18 (m, 1H), 1.02 (s, 9H), 0.93 (s, 3H), 0.89 (s, 9H), 0.82 (s, 3H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 172.1, 136.1, 134.3, 133.9,

129.9, 129.8, 127.8, 127.7, 104.1, 70.9, 69.9, 66.5, 59.6, 51.5, 48.3, 43.9, 43.5, 42.8, 36.3, 36.1, 27.0, 26.2, 20.5, 19.5, 18.5, 16.1, -5.00, -5.01; 125 MHz DEPT ($CDCl_3$) d CH_3: 51.5, 48.3, 27.0, 26.2, 20.5, 19.5, -5.00, -5.01, CH_2: 59.6, 43.9, 43.5, 36.3, 36.1, CH: 136.1, 129.9, 129.8, 127.8, 127.7, 70.9, 69.9, 66.5; IR (thin film) 3462, 2987, 2961, 2859, 1741, 1682, 1473, 1425, 1386, 1335, 1255, 1111, 735 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{37}H_{60}O_7Si_2Na$ *m/z* ($M + Na^+$): 695.3770. Found: 695.3793.



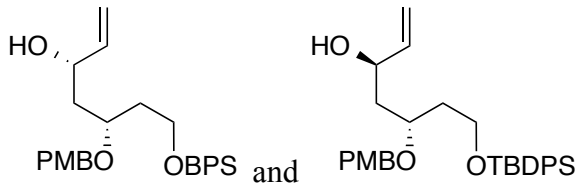
Preparation of methyl (*R*)-4-((2*S*,4*S*,6*S*)-6-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-methoxy-5,5-dimethyl-4-((phenoxycarbonothioyl) oxy)tetrahydro-2*H*-pyran-2-yl)-3-((*tert*-butyldiphenylsilyl)oxy)butanoate 3.9. To a stirring solution of alcohol **3.8** (45.1 mg, 0.067 mmol, 1.00 equiv), DMAP (1.60 mg, 0.013 mmol, 0.20 equiv), pyridine (67 μL) in CH_2Cl_2 (134 μL, 0.05 M) in a 5 mL reaction vial in an atmosphere of N_2, at 0 ^oC, was added phenyl thionocarbonyl chloride (46 μL, 0.34 mmol, 5.00 equiv) via syringe. The cold bath was removed and the reaction was stirred for 30 h. The reaction was poured into a separatory funnel containing CH_2Cl_2 (20 mL) and then the organic phase was washed with H_2O (2 × 10 mL), with brine (5 mL), dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a silica gel column (1 × 10 cm), eluting with 5% EtOAc/hexanes (100 mL), collecting 5 mL fractions. The fractions containing product (9-14) were combined and concentrated under reduced pressure to give the thionocarbonate **3.9** (39.0 mg, 72%) as yellow oil: R_f 0.52 (30%

EtOAc/ hexanes); $[\alpha]^{D}_{20}$ = +23.9 (*c* = 0.90, $CHCl_3$); 400 MHz ^{1}H NMR ($CDCl_3$) δ 7.72 – 7.64 (m, 4H), 7.47 – 7.36 (m, 8H), 7.32 – 7.28 (m, 1H), 7.10 – 7.08 (m, 2H), 5.41 (dd, *J* = 11.7, 5.0 Hz, 1H), 4.23 (m, 1H), 3.71 – 3.56 (m, 2H), 3.60 (s, 3H), 3.21 (m, 1H), 2.96 (s, 3H), 2.68 (dd, *J* = 14.8, 4.2 Hz, 1H), 2.57 (dd, *J* = 14.8, 7.7 Hz, 1H), 1.96 – 1.67 (m, 4H), 1.53 (td, *J* = 14.1, 3.6 Hz, 1H), 1.27 – 1.17 (m, 1H), 1.03 (s, 9H), 0.96 (s, 3H), 0.95 (s, 3H), 0.89 (s, 9H), 0.82 (s, 3H), 0.04 (s, 6H); 125 MHz ^{13}C NMR ($CDCl_3$) δ 194.6, 172.0, 153.5, 136.1, 136.0, 134.2, 133.8, 130.0, 130.0, 129.7, 127.8, 127.8, 126.7, 122.1, 104.3, 85.3, 70.9, 69.9, 66.2, 59.4, 51.6, 48.6, 43.8, 43.6, 42.4, 35.9, 31.7, 29.9, 27.1, 27.0, 26.1, 20.4, 19.4, 18.5, 17.8, -5.11, -5.13; 125 MHz DEPT ($CDCl_3$) d CH_3: 51.6, 48.6, 27.0, 26.1, 20.4, 17.8, -5.11, -5.13, CH_2: 59.4, 43.8, 43.6, 35.9, 31.7, CH: 136.1, 136.0, 130.0, 130.0, 129.7, 127.8, 127.8, 126.7, 122.1, 85.3, 69.9, 66.2; IR (thin film) 3458, 2985, 2961, 2859, 1741, 1678, 1473, 1425, 1386, 1241, 1111, 701 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{44}H_{64}O_8SSi_2Na$ ($M + Na^+$): 831.3752. Found: 831.3746.



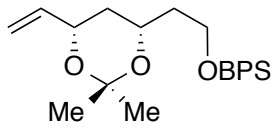
Preparation of methyl (*R*)-4-((2*S*,6*S*)-6-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-methoxy-5,5-dimethyltetrahydro-2*H*-pyran-2-yl)-3-((*tert*-butyldiphenylsilyl)oxy) butanoate 3.10. Thionocarbonate **3.9** (25 mg, 0.031 mmol, 1 equiv) was dissolved in benzene (310 μL, 0.1 M) in a 5 mL round-bottom flask. In another 5 mL round-bottom flask containing benzene (500 μL) were added Bu_3SnH (42 μL, 0.154 mmol, 5 equiv) via syringe and AIBN (2.5 mg, 0.015 mmol, 0.5 equiv) in one portion. A portion of this solution (100 μL) was added into the reaction flask containing the thionocarbonate and

heated to 90 °C. The rest of the AIBN/Bu_3SnH solution was added slowly to the reaction mixture via syringe pump (0.5 mL/h). After 1.5 h, the reaction was cooled to rt and then quenched by the addition of water. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 15% EtOAc/hexanes (400 mL) collecting in 5 mL fractions. The fractions containing product (18-25) were combined and concentrated under reduced pressure to give the product **3.10** (19 mg, 94%) as colorless oil: R_f = 0.53 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +18.2 (*c* = 0.120, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.76-7.61 (m, 4H), 7.48-7.33 (m, 6H), 4.24 (ddd, *J* = 12.2, 8.3, 3.9 Hz, 1H), 3.67 (dd, *J* = 18.1, 9.8 Hz, 1H), 3.59 (s, 3H), 3.55 (dd, *J* = 15.6, 8.3 Hz, 1H), 3.13 (m, 1H), 2.96 (s, 3H), 2.71 (dd, *J* = 14.6, 3.9 Hz, 1H), 2.54 (dd, *J* = 14.6, 8.3 Hz, 1H), 1.82 (m, 2H), 1.7 (m, 2H), 1.49 (m, 1H), 1.40-1.18 (m, 3H), 1.02 (s, 9H), 0.92 (s, 3H), 0.89 (s, 9H), 0.80 (s, 3H), 0.04 (s, 6H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 172.3, 136.0, 129.8, 129.8, 127.7, 102.7, 70.1, 67.7, 59.7, 51.6, 48.3, 44.2, 43.6, 36.5, 36.3, 34.8, 27.8, 27.0, 26.2, 25.8, 23.9, 19.5, 18.5, -5.0, -5.1; 125 MHz DEPT ($CDCl_3$) CH_3 δ 51.6, 48.3, 27.0, 26.2, 25.8, 24.0, -5.0; CH_2 δ 59.7, 44.2, 43.6, 36.3, 34.8, 27.8; CH δ 136.0, 129.8, 127.7, 70.1, 67.7; IR (neat) 3069, 2928, 2840, 1611, 1523, 1455, 1370, 1352, 1246, 1118, 1001, 900, 819 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{37}H_{60}O_6Si_2Na$ ($M + Na^+$): 679.382. Found: 679.3834.



Preparation of (3*S*,5*S*)-7-((*tert*-butyldiphenylsilyl)oxy)-5-((4-methoxybenzyl)oxy)hept-1-en-3-ol 3.26 and (3*R*,5*S*)-7-((*tert*-butyldiphenylsilyl)oxy)-5-((4-methoxybenzyl)oxy)hept-1-en-3-ol 3.19. Aldehyde **3.20** (313 mg, 0.656 mmol, 1.0 equiv) was dissolved in toluene (6.50 mL, 0.1 M) in a 15 mL round-bottom flask and cooled to -78 ^{o}C. After stirring for 10 min, a solution of Me_2AlCl (1.10 mL, 3.00 M in toluene, 3.28 mmol, 5.0 equiv) was added to the solution at -78 ^{o}C. In the meantime, a separate 5 mL round-bottom flask was charged with vinyl magnesium bromide (2.00 mL, 1.00 M in THF, 1.97 mmol, 3.0 equiv); the solvent was evaporated to near dryness under vacuum (0.1 mm of Hg) over about 30 min (complete dryness is avoided), and replaced with 2 mL of toluene. After 15 min at -78 ^{o}C, the vinyl magnesium bromide solution in toluene was added to the reaction mixture via cannula, dropwise over 5 min. The reaction mixture was allowed to stir for 2 h at -78 ^{o}C and then quenched by the addition of a saturated Rochelle's salt solution (3 mL). The cold bath was removed and the reaction mixture was then allowed to slowly reach rt. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 3 × 20 cm silica gel column, eluting with 15% EtOAc/hexanes (200 mL), then 20% EtOAc/hexanes (600 mL), collecting 5 mL fractions. The minor fractions containing product (35-40) were combined and concentrated under reduced pressure to give the minor diastereomer **3.19** (30 mg, 9%) as colorless oil. The major fractions containing product (45-85) were combined and concentrated under reduced pressure to give the major product **3.26** (258 mg, 78%) as colorless oil. Analytical data for the major diastereomer **3.26**: R_f = 0.33

(30% EtOAc/hexanes); $[\alpha]^{20}_{D}$ = +2.2 (*c* = 0.700, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.47- 7.36 (m, 6H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.81 (ddd, *J* = 16.6, 10.4, 5.7 Hz, 1H), 5.23 (d, *J* = 17.1 Hz, 1H), 5.07 (d, *J* = 10.4 Hz, 1H), 4.55-4.33 (ABq, *J* = 10.9 Hz, Δν = 76.3 Hz, 2H), 4.25 (m, 1H), 3.91-3.84 (m, 1H), 3.80 (s, 3H), 3.80 (m, 1H), 3.78-3.71 (m, 1H), 3.32 (s, 1H), 2.01-1.92 (m, 1H), 1.81-1.65 (m, 3H), 1.07 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 159.6, 141.1, 136.2, 135.9, 135.6, 135.3, 133.9, 130.3, 130.1, 129.7, 129.6, 128.3, 127.6, 114.8, 114.2, 113.9, 113.5, 76.6, 73.9, 69.9, 60.5, 55.5, 41.9, 41.8, 36.9, 27.2, 27.0, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.0; CH_2 δ 113.9, 69.9, 60.5, 41.8, 36.9; CH δ 141.1, 135.6, 129.6, 129.7, 127.6, 113.9, 73.9, 69.9; IR (neat) 3069, 2928, 2840, 1611, 1523, 1455, 1370, 1352, 1246, 1118, 1001, 900, 819 cm^{-1}; HRMS (EI) Calcd for $C_{31}H_{40}O_4SiNa$ ($M + Na^+$): 527.2588. Found: 527.2601. Analytical data for the minor diastereomer **3.19**: R_f = 0.35 (30% EtOAc/hexanes); $[\alpha]^{20}_{D}$ = +9.8 (*c* = 0.680, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.65 (m, 4H), 7.48- 7.37 (m, 6H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.87 (ddd, *J* = 16.9, 10.5, 5.3 Hz, 1H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.11 (d, *J* = 10.5 Hz, 1H), 4.46 (ABq, *J* = 11.0 Hz, Δν = 16.9 Hz, 2H), 4.38 (m, 1H), 4.00-3.94 (m, 1H), 3.81 (s, 3H), 3.80-3.72 (m, 2H), 2.91 (s, 1H), 2.03-1.93 (m, 1H), 1.84-1.70 (m, 3H), 1.08 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 159.5, 141.2, 135.8, 134.0, 134.0, 133.9, 130.5, 129.9, 129.8, 129.7, 128.0, 127.9, 114.1, 114.1, 77.4, 74.1, 71.2, 70.1, 60.7, 55.5, 40.4, 36.8, 27.3, 27.1, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.3, 26.8; CH_2 δ 113.9, 71.0, 60.5, 40.2, 36.6; CH δ 141.2, 135.8, 129.6, 129.5, 127.7, 113.9, 73.9, 69.9; IR (neat) 3069, 2928, 2840, 1611, 1523, 1455, 1370, 1352, 1246, 1118, 1001, 900, 819 cm^{-1}; HRMS (EI) Calcd for $C_{31}H_{40}O_4SiNa$ ($M + Na^+$): 527.2588. Found: 527.2603.



Preparation of *tert*-butyl(2-((4*S*,6*S*)-2,2-dimethyl-6-vinyl-1,3-dioxan-4-yl)ethoxy)diphenylsilane 3.27. To a solution of the major diastereomer **3.26** (30.0 mg, 0.059 mmol, 1.0 equiv) in CH_2Cl_2 (600 μL, 0.1 M) in a 5 mL round-bottom flask was added pyridine (24 μL, 0.295 mmol, 5.0 equiv), DMAP (~1 mg, 0.006 mmol, 0.1 equiv), and Ac_2O (17 μL, 0.178 mmol, 3.0 equiv) sequentially at rt. The mixture was stirred for 2 h and then quenched by the addition of saturated $NaHCO_3$ solution (2 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified using a silica gel column (1 × 10 cm), eluting with 10% EtOAc/hexanes (200 mL), then 15% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (25-45) were combined and concentrated under reduced pressure to give the acetate product as colorless oil. The acetate was taken forward for the next step.

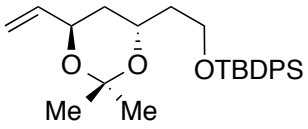
The acetate (assumed to be 0.059 mmol) was taken up in CH_2Cl_2 (5.9 mL, 0.01 M) in a 15 mL round-bottom flask. *t*BuOH (118 μL, 0.5 M) and pH 7 buffer (1.2 mL, 0.1 M, 0.118 mmol, 2 equiv) were added to the reaction mixture at rt. DDQ (53 mg, 0.236 mmol, 4 equiv) was added in one portion and the reaction was stirred vigorously for 1.5 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (3 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4,

filtered, and concentrated under reduced pressure. This alcohol was carried forward directly for the next step.

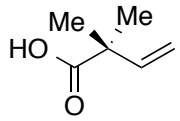
The acetate (assumed to be 0.059 mmol) was dissolved in MeOH (5.00 mL, 0.1 M) in a 15 mL round-bottom flask at rt. K_2CO_3 (41 mg, 0.295 mmol, 5 equiv) was then added in one portion. The reaction mixture was stirred vigorously for 1.5 h and then quenched by the addition of saturated $NaHCO_3$ solution (3 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a 1 × 10 cm silica gel column, eluting with 25% EtOAc/hexanes (400 mL), collecting 9 mL fractions. The fractions containing product (20-32) were combined and concentrated under reduced pressure to give the acetate product as colorless oil. This oil was then directly taken to the next step.

The diol (assumed to be 0.059 mmol) was taken up in CH_2Cl_2 (1.2 mL, 0.05 M) in a 10 mL round-bottom flask at rt. 2-methoxypropene (28 μL, 0.295 mmol, 5 equiv) was then added dropwise via a syringe followed by PPTS (~1 crystal) in one portion. The reaction was stirred for 1 h and then quenched by the addition of saturated $NaHCO_3$ solution (3 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 5% EtOAc/hexanes (400 mL) collecting 5 mL fractions. The fractions containing product (12-16) was combined and concentrated under reduced pressure to give the acetate

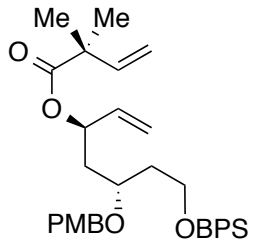
product as colorless oil: R_f = 0.58 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +3.8 (*c* = 0.210, $CHCl_3$); 500 MHz ^1H NMR ($CDCl_3$) δ 7.71-7.66 (m, 4H), 7.46- 7.36 (m, 6H), 5.83 (ddd, *J* = 16.6, 10.5, 5.9 Hz, 1H), 5.26 (d, *J* = 17.1 Hz, 1H), 5.13 (d, *J* = 10.5 Hz, 1H), 4.35 (m, 1H), 4.18 (m, 1H), 3.86 (m, 1H), 3.73 (m, 1H), 1.72 (m, 2H), 1.49 (s, 3H), 1.43 (m, 4H), 1.07 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 139.1, 135.8, 134.2, 134.1, 129.8, 129.8, 127.8, 127.8, 115.4, 98.8, 70.5, 65.6, 59.8, 39.5, 37.2, 30.5, 27.1, 20.0, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 30.5, 26.8, 20.0; CH_2 δ 115.1, 59.6, 39.3, 36.9; CH δ 138.8, 135.5, 135.5, 129.6, 129.5, 127.6, 127.5, 70.2, 65.3; IR (neat) 3069, 2928, 2840, 1611, 1452, 1305, 1348, 1122, 989, 889, 823 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{26}H_{36}O_3SiNa$ ($M + Na^+$): 447.2326. Found: 447.2340.



Preparation of *tert*-butyl(2-((4*S*,6*R*)-2,2-dimethyl-6-vinyl-1,3-dioxan-4-yl)ethoxy)diphenylsilane 3.19. Same procedure as **3.26**: R_f = 0.58 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +5.6 (*c* = 0.210, $CHCl_3$); 500 MHz ^1H NMR ($CDCl_3$) δ 7.72-7.65 (m, 4H), 7.46- 7.36 (m, 6H), 5.91 (ddd, *J* = 17.1, 10.5, 5.9 Hz, 1H), 5.23 (d, *J* = 17.1 Hz, 1H), 5.14 (d, *J* = 10.5 Hz, 1H), 4.35 (m, 1H), 4.16 (m, 1H), 3.82 (m, 1H), 3.73 (m, 1H), 1.84-1.65 (m, 2H), 1.40 (s, 3H), 1.38 (m, 4H), 1.06 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 139.1, 135.8, 135.8, 134.2, 134.1, 129.8, 129.8, 127.8, 115.2, 100.5, 68.2, 63.4, 60.2, 39.2, 37.7, 27.1, 25.8, 25.1, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 27.1, 25.8, 25.1; CH_2 δ 115.3, 60.3, 39.2, 37.8; CH δ 139.1, 135.8, 129.8, 127.9, 68.2, 63.4; IR (neat) 3069, 2928, 2840, 1611, 1452, 1305, 1348, 1122, 989, 889, 823 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{26}H_{36}O_3SiNa$ ($M + Na^+$): 447.2326. Found: 447.2338.

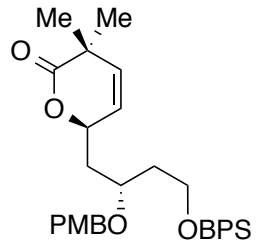


Preparation of 2,2-dimethylbut-3-enoic acid 3.18. A solution of *n*BuLi (88 mL, 2.5 M in hexanes, 220.6 mmol, 2.2 equiv) in THF (88 mL, 2.5 M) was cooled to -78 ^{o}C under nitrogen atmosphere. Diethylamine (21.8 mL, 210.6 mmol, 2.1 equiv) was added to the solution and the solution was stirred for 15 min at 0 ^{o}C, and then cooled to -78 ^{o}C again. The tiglic acid (10.0 g, 100.3 mmol, 1.0 equiv) was then added slowly during 5 min to the stirring solution and the resulting yellow solution was stirred for 30 min at 0 ^{o}C, and then cooled down again to -78 ^{o}C. Me_2SO_4 (9.5 mL, 100.3 mmol, 1.0 equiv) in THF (200 mL, 0.5 M) was added slowly and the solution was stirred for another 30 min at 0 ^{o}C and then slowly warmed up to rt over 1 h. The reaction was then quenched with water (100 mL) and the reaction mixture was extracted with Et_2O (3 × 50 mL). The aqueous layer was acidified with ice-cold solution of conc. HCl and then extracted with EtOAc (3 × 20 mL), and the combined organic layer was dried with Na_2SO_4 and evaporation of the solvent gave the crude acid reaction mixture. Purification was accomplished by flash chromatography on a silica gel column (3 × 25 cm), eluting with 40-50% EtOAc/hexanes (1000 mL) collecting 9 mL fractions. The fractions containing product (19-40) were combined and concentrated under reduced pressure to give the acetate product (6.0 g, 53%) as colorless oil: R_f = 0.40 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 6.06 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.16 (d, *J* = 17.6 Hz, 1H), 5.11 (d, *J* = 10.8 Hz, 1H), 1.34 (s, 6H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 183.5, 142.1, 113.6, 44.9, 24.6.



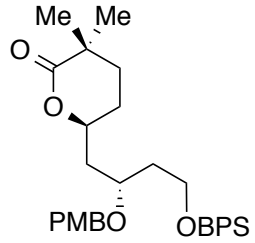
Preparation of (3*R*,5*S*)-7-((*tert*-butyldiphenylsilyl)oxy)-5-((4-methoxybenzyl)oxy)hept-1-en-3-yl 2,2-dimethylbut-3-enoate 3.17. To a solution of the alcohol **3.26** (324 mg, 0.642 mmol, 1.0 equiv) in THF (13 mL, 0.05 M) in a 25 mL round-bottom flask at rt was added PPh_3 (842 mg, 3.21 mmol, 5 equiv) and DEAD (505 μL, 3.21 mmol, 5 equiv). The acid **3.18** (293 mg, 2.57 mmol, 4 equiv) was dissolved in THF (1 mL) in a separate flask and added to the reaction mixture via cannula. The reaction mixture was allowed to stir for 2 h at rt and then quenched by the addition of saturated $NaHCO_3$ solution (3 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (3 × 15 cm), eluting with 10% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (15-25) were combined and concentrated under reduced pressure to give the ester **3.17** (323 mg, 84%) as colorless oil: R_f = 0.53 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +8.2 (*c* = 0.630, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.75-7.65 (m, 4H), 7.48-7.36 (m, 6H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.06 (dd, *J* = 17.5, 10.6 Hz, 1H), 5.79 (ddd, *J* = 16.9, 10.6, 5.9 Hz, 1H), 5.51 (m, 1H), 5.24 (d, *J* = 17.2 Hz, 1H), 5.10 (m, 4H), 4.44-4.29 (ABq, *J* = 10.6 Hz, Δν = 51.0 Hz, 2H), 3.87-3.65 (m, 3H), 3.80 (s, 3H), 1.90-1.74 (m, 1H), 1.33 (s, 3H), 1.32 (s, 3H), 1.08 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 175.4, 159.4, 142.7, 137.0, 135.8, 133.9, 130.8, 129.8, 129.5, 127.9, 127.9,

116.1, 114.0, 113.9, 113.2, 72.6, 71.9, 71.8, 60.6, 55.5, 45.2, 40.5, 37.8, 27.1, 24.8, 24.7, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1, 24.8, 24.7; CH_2 δ 116.1, 114.0, 72.6, 71.8, 41.8, 36.9; CH δ 142.5, 137.0, 129.8, 129.5, 127.9, 127.9, 113.9, 72.6, 71.9; IR (neat) 3056, 2936, 2834, 1736, 1619, 1447, 1370, 1354, 1246, 1224, 1118, 823 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{37}H_{48}O_5SiNa$ ($M + Na^+$): 623.3163. Found: 623.3174.



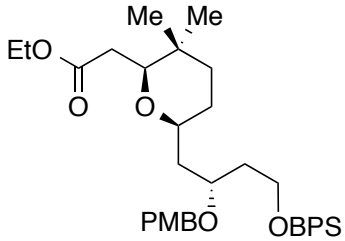
Preparation of (*R*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyl-3,6-dihydro-2*H*-pyran-2-one 3.31. The ester **3.17** (450 mg, 0.749 mmol, 1 equiv) was dissolved in degassed CH_2Cl_2 (150 mL, 0.005 M). CH_2Cl_2 was degassed with a steady flow of N_2 for 30 min prior to use. $Ti(OiPr)_4$ solution (3.7 mL, 1 M in CH_2Cl_2, 3.74 mmol, 5 equiv) was then added dropwise to the reaction mixture. It was then heated at reflux for 1 h under N_2 atmosphere. Grubbs' 2nd-generation catalyst **3.30** (127 mg, 0.150 mmol, 0.20 equiv) was then added in one portion and heated at reflux for another 12 h. The reaction mixture was then passed through a plug of Celite®, and then the solution was stirred in an open Erlenmeyer flask with activated charcoal overnight. The solution was then filtered through a plug of Celite® and then treated with H_2O_2 for 2 h until effervescence stopped and the solution became colorless. The excess H_2O_2 (10 mL) was then quenched by the addition of saturated aqueous NaS_2O_3 solution (10 mL). The phases were separated and the aqueous layer was then extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure.

Purification was accomplished by flash chromatography on a silica gel column (3 × 15 cm), eluting with 10% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (15-25) were combined and concentrated under reduced pressure to give the ester **3.17** (412 mg, 96%) as colorless oil: R_f = 0.35 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +10.5 (*c* = 0.500, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.66 (m, 4H), 7.47- 7.36 (m, 6H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.64 (s, 1H), 5.14 (dd, *J* = 6.2, 5.7 Hz, 1H), 4.54-4.40 (ABq, *J* = 10.8 Hz, Δν = 40.9 Hz, 2H), 4.14 (dd, *J* = 14.4, 7.2 Hz, 1H), 4.0 (m, 1H), 3.80 (s, 3H), 3.80 (m, 2H), 1.92-1.78 (m, 4H), 1.35 (s, 3H), 1.32 (s, 3H), 1.08 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 175.3, 159.4, 135.8, 133.9, 133.9, 132.7, 130.9, 129.8, 129.7, 127.9, 125.0, 114.0, 76.3, 72.8, 72.0, 60.5, 55.5, 42.9, 38.0, 37.6, 28.1, 27.1, 26.9, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.1, 24.8, 24.7; CH_2 δ 116.1, 114.0, 72.6, 71.8, 41.8, 36.9; CH δ 135.8, 132.7, 129.8, 129.7, 125.0, 114.0, 76.3, 72.8; IR (neat) 3056, 2936, 1735, 1615, 1455, 1370, 1354, 1246, 1112, 816 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{35}H_{44}O_5SiNa$ (M + Na^+): 595.2848. Found: 595.2856.



Preparation of (*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-one 3.16. To a solution of the olefin **3.31** (46 mg, 0.080 mmol, 1 equiv) in EtOAc (800 µL, 0.1 M) under an atmosphere of H_2 (balloon) at 0 ^{o}C was added Adams' catalyst (2 mg, 10 wt%) quickly in one portion. The reaction was closely monitored by TLC for any over-reduction. The

balloon was removed as soon as the starting material was consumed. The mixture was then filtered through a plug of Celite®, washed with EtOAc (3 × 10 mL), and then evaporated under reduced pressure. The crude mixture was purified by flash chromatography on a silica gel column (1 × 10 cm), eluting with 15-20% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (13-17) were combined and concentrated under reduced pressure to give the lactone **3.16** (34 mg, 74%) as colorless oil: R_f = 0.30 (25% EtOAc/hexanes); $[\alpha]^{20}_D$ = +8.3 (*c* = 0.100, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.47- 7.36 (m, 6H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 4.45-4.35 (ABq, *J* = 11.2 Hz, Δν = 39.9 Hz, 2H), 3.86-3.73 (m, 3H), 3.80 (s, 3H), 2.02 (ddd, *J* = 13.7, 6.8, 6.8 Hz, 1H), 1.89 (ddd, *J* = 14.2, 6.4, 6.4 Hz, 1H), 1.80 (m, 1H), 1.73 (m, 1H), 1.70-1.55 (m, 4H), 1.30 (s, 3H), 1.26 (s, 3H), 1.07 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 177.5, 159.4, 135.8, 133.9, 130.8, 129.9, 129.7, 127.9, 127.9, 113.9, 79.0, 72.0, 70.6, 60.5, 55.5, 40.8, 38.3, 36.8, 34.7, 28.1, 28.0, 27.1, 26.2, 19.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 28.1, 28.0, 27.1; CH_2 δ 70.6, 60.5, 40.8, 36.8, 34.7, 26.2; CH δ 135.8, 129.9, 129.7, 127.9, 127.9, 114.0, 79.0, 72.0; IR (neat) 3065, 2936, 1736, 1612, 1455, 1370, 1352, 1246, 1112, 816 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{35}H_{46}O_5SiNa$ ($M + Na^+$): 597.3007. Found: 597.3022.

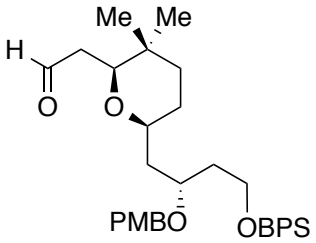


Preparation of ethyl 2-((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)acetate 3.15. A solution of *i*Pr_2NH (166 μL, 1.19 mmol, 25.1 equiv) in THF (12 mL, 0.1 M) was cooled

to -78 ^{o}C. *n*BuLi (472 μL, 2.5 M in hexanes, 1.18 mmol, 25 equiv) was then added dropwise via syringe. The solution was then warmed to 0 ^{o}C and stirred for 30 min. The solution was then cooled again to -78 ^{o}C. EtOAc (116 μL, 1.18 mmol, 25 equiv) was then added dropwise via syringe slowly over 2 min. The reaction mixture was then stirred for another 45 min. To this solution was added lactone **3.16** (27.2 mg, 0.047 mmol, 1 equiv) in THF (1 mL) slowly down the side of the flask via cannula. Another 1 mL of THF was used to wash the flask and then transferred to the reaction mixture. The reaction was allowed to stir for 1 h and then quenched by the addition of saturated NH_4Cl solution (10 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude mixture was then taken directly to the next step.

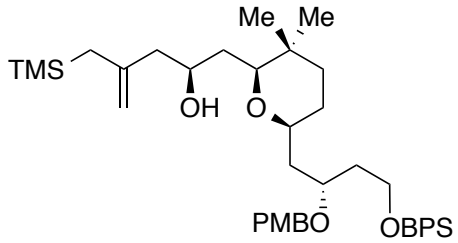
The hemiketal (assumed to be 0.047 mmol) was dissolved in CH_2Cl_2 and cooled to -78 ^{o}C. Et_3SiH (151 μL, 0.946, 20 equiv) was added to the reaction mixture and stirred for 10 min. To this solution was added TMSOTf (52 μL, 0.052, 1.1 equiv) dropwise over 2 min. The reaction mixture was stirred at -78 ^{o}C for 1.5 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification was accomplished by flash chromatography on a 1 × 10 cm silica gel column, eluting with 10% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (13-24) were combined and concentrated under reduced pressure to give the ester **3.17** (25 mg, 84%) as colorless oil: R_f = 0.52 (25%

EtOAc/hexanes); $[\alpha]^{20}_D$ = +6.5 (*c* = 0.500, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.65 (m, 4H), 7.48- 7.35 (m, 6H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.86 (d, *J* = 8.2 Hz, 2H), 4.40 (ABq, *J* = 10.9 Hz, Δν = 17.3 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 3.80 (m, 3H), 3.65 (dd, *J* = 9.8, 1.7 Hz, 1H), 3.55 (m, 1H), 2.46 (m, 1H), 2.36 (m, 1H), 1.79 (ddd, *J* = 6.2, 6.2, 6.2 Hz, 2H), 1.59 (m, 2H), 1.52-1.34 (m, 4H), 1.20 (m, 3H), 1.07 (s, 9H), 0.93 (s, 3H), 0.88 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 172.6, 159.3, 135.8, 134.2, 134.2, 131.6, 129.8, 129.7, 127.8, 113.9, 82.0, 75.0, 73.2, 72.3, 60.9, 60.5, 55.5, 42.6, 39.5, 38.3, 36.3, 32.4, 29.2, 27.5, 27.1, 19.4, 19.4, 14.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.5, 27.1, 19.4, 14.5; CH_2 δ 72.3, 60.9, 60.5, 42.6, 39.5, 38.3, 36.3, 29.2; CH δ 135.8, 129.7, 129.7, 127.8, 113.9, 82.0, 75.0, 73.2; IR (neat) 3056, 2936, 1750, 1615, 1455, 1370, 1354, 1246, 1050, 816 cm^{-1}; HRMS (EI) Calcd for $C_{39}H_{54}O_6Si$ (M + Na^+): 669.3582. Found: 669.3595.



Preparation of 2-((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)acetaldehyde 3.14. The ester **3.17** (300 mg, 0.464 mmol, 1 equiv) was dissolved in CH_2Cl_2 (5 mL, 0.1 M) in a 15 mL round-bottom flask. The solution was then cooled to -78 ^{o}C and stirred for 10-15 min. DIBAL-H (650 μL, 1.5 M in toluene, 0.974, 2.1 equiv) was added slowly down the side of the flask over 20 min by means of syringe pump. The reaction mixture was stirred for 1.5 h and then quenched by the addition of acetone (1 mL) and then stirred for another 10 min. Rochelle's salt solution (5 mL) was added and then the reaction

mixture was slowly warmed up to rt. The phases were separated and the aqueous layer was extracted by CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (3 × 15 cm), eluting with 5-15% EtOAc/hexanes (800 mL), collecting 5 mL fractions. The fractions containing product (31-43) were combined and concentrated under reduced pressure to give the ester **3.14** (243 mg, 87%) as colorless oil: R_f = 0.45 (20% EtOAc/hexanes); $[\alpha]^{20}_D$ = +4.1 (*c* = 0.500, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.68 (t, *J* = 2.0 Hz, 1H), 7.71-7.64 (m, 4H), 7.46- 7.35 (m, 6H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.46-4.31 (ABq, *J* = 11.2 Hz, Δν = 51.3 Hz, 2H), 3.81-3.71 (m, 2H), 3.80 (s, 3H), 3.56 (dd, *J* = 10.3, 2.9 Hz, 1H), 3.53 (m, 1H), 3.49 (m, 1H), 2.39 (m, 2H), 1.78 (m, 2H), 1.66-1.35 (m, 6H), 1.06 (s, 9H), 0.93 (s, 3H), 0.84 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 202.6, 159.3, 135.8, 134.1, 131.4, 129.8, 129.6, 127.9, 127.8, 114.0, 80.7, 75.1, 72.8, 71.8, 66.0, 60.7, 55.5, 44.4, 42.4, 39.3, 37.9, 32.4, 29.1, 27.4, 27.1, 19.4, 19.4, 15.5; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.5, 27.4, 27.1, 19.4; CH_2 δ 71.8, 60.7, 44.4, 42.4, 39.3, 37.9, 29.1; CH δ 202.6, 135.8, 129.8, 129.7, 129.6, 127.8, 114.0, 80.7, 75.1, 72.8; IR (neat) 3056, 2826, 2730, 1734, 1615, 1455, 1370, 1354, 1246, 1050, 816 cm^{-1}; HRMS (EI) Calcd for $C_{37}H_{50}O_5SiNa$ (M): 625.3320. Found: 625.3329.

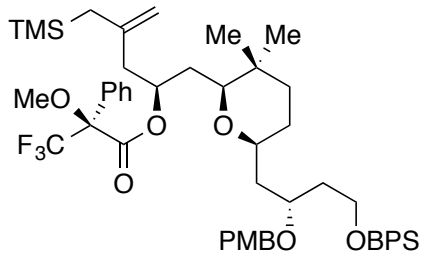


Preparation of (*S*)-1-((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)-4-

((trimethylsilyl)methyl)pent-4-en-2-ol 3.12. A 50 mL round-bottom flask was charged with *N*,*N*'-((1*S*,2*S*)-1,2-diphenylethane-1,2-diyl)bis(4-methylbenzenesulfonamide) **3.33** (100 mg, 0.192 mmol, 3 equiv) and a stir bar. The flask was heated for 18 h at 100 ^{o}C under vacuum (0.1 mm of Hg). The dry powder was then cooled to rt and flushed with Ar from Schlenk line. CH_2Cl_2 (274 μL, 0.7 M) was added to the flask via syringe and stirred for 1 h. The suspension was then cooled down to 0 ^{o}C. BBr_3 (18 μL, 0.192 mmol, 3 equiv) was added dropwise via syringe, and then the solution was stirred for another 1 h at rt. Next, the flask was connected to the vacuum line and the solvent was removed under vacuum for about 30 min This should also remove volatile bromine from the reaction mixture. A separate 25 mL pear shaped flask was charged with 2,6-di-tert-butyl-4-methylpyridine (39.4 mg, 0.192 mmol, 3 equiv) followed by trimethyl(2-((tributylstannyl)methyl)allyl)silane **3.34** (77 μL, 0.192 mmol, 3 equiv) and was flushed with Ar 2-3 times and then left connected with Ar Schlenk line. CH_2Cl_2 (2 mL, 0.1 M) was added to the mixture via syringe and cannulated into the reaction mixture. This mixture was then stirred for another 16 h at rt. After 16 h, the solution was cooled down to -78 ^{o}C.

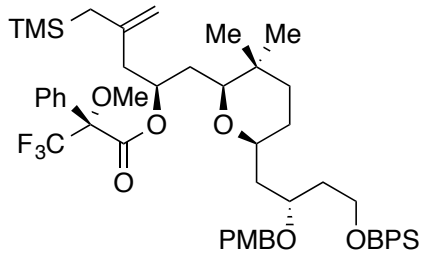
The aldehyde **3.14** (271 mg, 0.449 mmol, 1 equiv) was dissolved in CH_2Cl_2 (9 mL, 0.05 M) in a 15 mL round-bottom flask. This solution was then cooled down to -78 ^{o}C and then cannulated to the flask containing the reagent over 15 min The reaction mixture was then stirred for another 2 h before being quenched by the addition of aqueous pH 7 buffer solution (10 mL, 0.1 M) and warmed to rt. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was evaporated under reduced

pressure to about ⅓ of the original volume. A mixture of Et_2O/hexanes (2:1, 20 mL) was added and the *N*,*N*'-((1*S*,2*S*)-1,2-diphenylethane-1,2-diyl)bis(4-methylbenzenesulfonamide) precipitated and was recovered by filtration. The filtrate was concentrated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 5% EtOAc/hexanes (400 mL), collecting 5 mL fractions. The fractions containing product (23-41) were combined and concentrated under reduced pressure to give the ester **3.12** (35.5 mg, 76%) as colorless oil: R_f = 0.55 (20% EtOAc/hexanes); $[\alpha]^{20}_D$ = +17.1 (*c* = 0.500, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.71-7.64 (m, 4H), 7.45- 7.35 (m, 6H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 4.66 (s, 1H), 4.62 (s, 1H), 4.41 (ABq, *J* = 10.8 Hz, Δν = 18.1 Hz, 2H), 4.00-3.92 (m, 2H), 3.80 (s, 3H), 3.81-3.69 (m, 1H), 3.54 (m, 1H), 3.27 (m, 1H), 2.23 (dd, J = 14.2, 6.4 Hz, 1H), 2.04 (dd, *J* = 14.2, 6.8 Hz, 1H), 1.85-1.75 (m, 2H), 1.74-1.60 (m, 4H), 1.50-1.34 (m, 5H), 1.33-1.22 (m, 2H), 1.06 (s, 9H), 0.92 (s, 3H), 0.82 (s, 3H), 0.03 (s, 9H); IR (neat) 3056, 2826, 2730, 1734, 1615, 1455, 1370, 1354, 1246, 1050, 816 cm^{-1}; HRMS (EI) Calcd for $C_{44}H_{66}O_5Si_2$ ($M + Na^+$): 753.4341. Found: 753.4354.

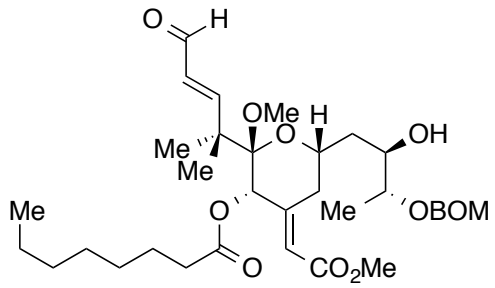


Preparation of (*S*)-1-((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)-4-((trimethylsilyl)methyl)pent-4-en-2-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 3.38. To a solution of the alcohol **3.12** (12.5 mg, 0.017 mmol, 1

equiv) in CH_2Cl_2 (171 μL, 0.1 M) in a 5 mL vial was added DMAP (1 mg, 0.007 mmol, 0.4 equiv), EDCI•HCl (9.8 mg, 0.051 mmol, 3 equiv) and (*S*)-MTPA (8 mg, 0.034 mmol, 2 equiv) at rt. The reaction mixture was stirred for 9 d and then quenched by the addition of saturated $NaHCO_3$ solution (2 mL). The phases were separated and the aqueous layer was extracted by CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 2.5-5% EtOAc/hexanes (200 mL), collecting 5 mL fractions. The fractions containing product (15-19) were combined and concentrated under reduced pressure to give the ester **3.38** (12.6 mg, 78%) as colorless oil: R_f = 0.55 (20% EtOAc/hexanes); $[\alpha]^{20}_D$ = +12.5 (*c* = 0.200, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.72-7.65 (m, 4H), 7.59-7.53 (m, 2H), 7.44- 7.33 (m, 9H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 5.52 (m, 1H), 4.56 (s, 1H), 4.50 (s, 1H), 4.38 (s, 2H), 3.83-3.75 (m, 3H), 3.79 (s, 3H), 3.54 (s, 3H), 3.42 (m, 1H), 3.13 (dd, *J* = 9.8, 1.5 Hz, 1H), 2.28 (m, 2H), 2.06 (m, 1H), 1.90-1.62 (m, 7H), 1.56 (m, 2H), 1.53-1.33 (m, 7H), 1.05 (s, 9H), 0.89 (s, 3H), 0.80 (s, 3H), -0.05 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 165.8, 159.2, 142.6, 135.8, 134.2, 134.2, 132.7, 131.4, 129.7, 129.6, 129.4, 128.4, 127.8, 113.9, 111.0, 82.0, 75.4, 74.2, 73.5, 71.4, 60.8, 55.6, 55.5, 42.5, 42.2, 39.7, 37.8, 34.6, 32.6, 29.9, 29.0, 27.5, 27.1, 26.8, 19.4, 19.2, -1.3; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.6, 55.5, 27.5, 27.1, 27.1, 19.2, -1.3; CH_2 δ 111.0, 71.3, 60.7, 42.4, 42.2, 39.6, 37.8, 34.6, 29.0, 26.8; CH δ 135.8, 129.7, 129.6, 129.4, 128.4, 127.8, 127.8, 113.9, 82.0, 75.4, 74.1, 73.5; IR (neat) 3056, 2936, 2859, 1765, 1615, 1590, 1473, 1370, 1354, 1246, 1050, 824, 816 cm^{-1}; HRMS (EI) Calcd for $C_{54}H_{73}F_3O_7Si_2Na$ *m/z* ($M + Na^+$): 969.4739. Found: 969.4746.

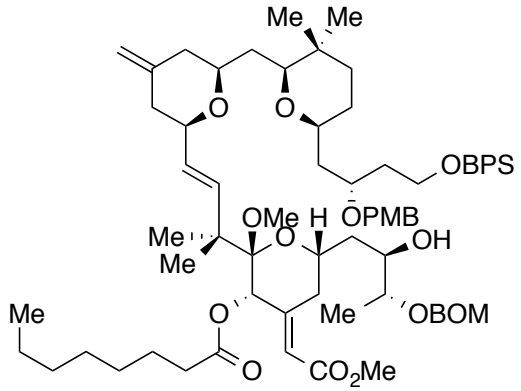


Preparation of (*S*)-1-((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)-4-((trimethylsilyl)methyl)pent-4-en-2-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 3.37. Same procedure as **3.38** with (*R*)-MTPA: R_f = 0.55 (20% EtOAc/hexanes); $[\alpha]^{20}_D$ = +2.5 (*c* = 0.020, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.59-7.53 (m, 2H), 7.45-7.32 (m, 9H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 5.54 (m, 1H), 4.66 (s, 1H), 4.60 (s, 1H), 4.38 (s, 2H), 3.85-3.74 (m, 3H), 3.79 (s, 3H), 3.53 (s, 3H), 3.38 (m, 1H), 3.07 (dd, *J* = 9.8, 1.5 Hz, 1H), 2.41-2.30 (m, 2H), 1.81-1.71 (m, 4H), 1.71-1.61 (m, 3H), 1.57-1.19 (m, 13H), 1.05 (s, 9H), 0.86 (s, 6H), 0.73 (s, 3H), -0.02 (s, 9H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 166.0, 159.2, 143.0, 135.8, 134.2, 134.2, 132.7, 131.4, 129.7, 129.6, 129.5, 129.4, 128.4, 127.8, 113.9, 110.8, 81.4, 75.2, 74.2, 73.5, 71.3, 60.8, 55.7, 55.5, 42.3, 42.2, 39.6, 37.9, 34.5, 32.6, 31.8, 29.9, 29.0, 27.4, 27.1, 26.9, 22.9, 19.4, 19.1, 14.3, 1.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.4, 55.2, 27.5, 27.2, 26.9, 18.9, 1.4; CH_2 δ 110.6, 71.0, 60.6, 42.0, 41.9, 39.4, 37.6, 34.2, 29.7, 28.7, 26.6; CH δ 135.6, 129.5, 129.4, 129.4, 129.2, 129.1, 128.2, 127.6, 113.7, 81.2, 74.9, 73.9, 73.2; IR (neat) 3056, 2936, 2859, 1765, 1615, 1590, 1455, 1370, 1354, 1246, 1050, 824, 816 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{54}H_{73}F_3O_7Si_2Na$ *m*/*z* ($M + Na^+$): 969.4739. Found: 969.4745.



Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-hydroxybutyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-2-((*E*)-2-methyl-5-oxopent-3-en-2-yl)tetrahydro-2*H*-pyran-3-yl octanoate 3.41. The TBS ether **3.11** (34.2 mg, 0.046 mmol, 1 equiv) was dissolved in THF/Py (9:1, 4.6 mL, 0.01 M) in a 10 mL plastic vial at rt. MeOH (500 μL), followed by 2.5 mL of HF•Py (20% of HF in Py), was added to the reaction mixture. After stirring for 12 h at rt, the reaction was judged incomplete by TLC analysis and an additional 1 mL of HF•Py was added to the reaction mixture. After another 24 h, the reaction was complete and cooled to 0 ^{o}C. Powdered $NaHCO_3$ was added slowly to the reaction mixture to quench until effervescence was complete. Water was added to the reaction mixture which was then warmed to rt. The phases were separated and the aqueous layer was extracted by CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 15-25% EtOAc/hexanes (600 mL), collecting 9 mL fractions. The fractions containing product (50-55) were combined and concentrated under reduced pressure to give the alcohol **3.41** (26.6 mg, 92%) as colorless oil: R_f = 0.40 (50% EtOAc/hexanes); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.50 (d, *J* = 7.3 Hz, 1H), 7.34-7.25 (m, 5H), 7.23 (d, *J* = 8.8 Hz, 2H), 5.89 (dd, *J* = 16.1, 7.8 Hz, 1H), 5.83 (s, 1H), 5.46 (s, 1H), 4.86-4.77 (m, 1H), 4.82 (ABq, *J* = 7.3 Hz, Δν = 23.5 Hz, 2H), 4.65-4.56 (m, 1H),

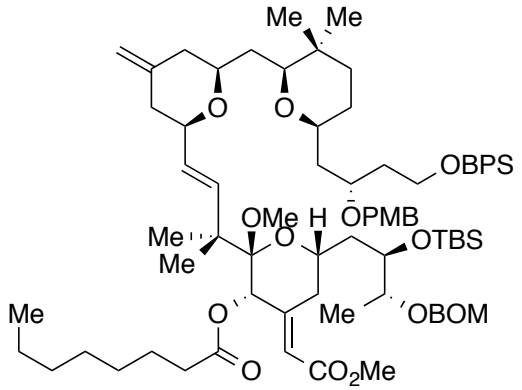
4.62 (ABq, J = 11.7 Hz, Δν = 20.5 Hz, 2H), 4.23 (td, J = 8.8, 2.9 Hz, 1H), 3.79 (m, 1H), 3.65 (s, 3H), 3.59 (m, 2H), 3.45 (m, 1H), 3.38 (s, 3H), 2.63 (s, 1H), 2.34 (t, J = 13.7 Hz, 1H), 2.19-2.02 (m, 2H), 1.69 (m, 2H), 1.50 (m, 2H), 1.33-1.17 (m, 12H), 1.15 (s, 3H), 1.11 (s, 3H), 0.83 (t, J = 7.3 Hz, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 194.7, 171.9, 167.0, 166.5, 151.9, 137.6, 128.7, 128.1, 128.0, 127.1, 102.6, 94.1, 71.4, 71.2, 70.2, 68.7, 51.5, 47.7, 39.9, 34.6, 33.1, 31.8, 29.9, 29.1, 29.0, 24.8, 23.9, 22.7, 22.1, 17.0, 14.2; IR (neat) 3056, 2936, 2859, 1735, 1615, 1590, 1473, 1370, 1354, 1246, 1050, 824, 816 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{35}H_{52}O_{10}Na$ (M + Na^+): 655.3452. Found: 655.3463.



Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-hydroxybutyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 3.42. To a solution of the β-hydroxyallyl silane **3.12** (16 mg, 0.023 mmol, 1.3 equiv) in Et_2O (1.1 mL, 0.02 M) in a 5 mL vial was added a solution of aldehyde **3.41** (11 mg, 0.017 mmol, 1 equiv) in Et_2O (250 μL) via cannula. Another 250 μL was used to transfer all the aldehyde to the reaction mixture. The mixture was then cooled to -78 ^{o}C and stirred for 10 min before TMSOTf (23 μL, 1 M in Et_2O, 0.023 mmol, 1.3 equiv) was added to the reaction mixture

slowly down the side of the flask. The resulting solution was stirred for 2 h and then quenched by addition of *i*Pr_2EtN (1 mL) and saturated aqueous $NaHCO_3$ solution (2 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (0.5 × 10 cm), eluting with 10-25% EtOAc/hexanes (1000 mL), collecting 5 mL fractions. The fractions containing product (112-123) were combined and concentrated under reduced pressure to give the alcohol **3.42** (16.6 mg, 75%) as colorless oil: R_f = 0.25 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +14.5 (*c* = 0.120, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.65 (m, 4H), 7.45-7.28 (m, 11H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.96 (d, *J* = 15.1 Hz, 1H), 5.87 (s, 1H), 5.57 (m, 1H), 5.42 (dd, *J* = 16.1, 5.9 Hz, 1H), 4.86 (ABq, *J* = 6.8 Hz, Δν = 24.5 Hz, 2H), 4.65 (ABq, *J* = 11.7 Hz, Δν = 18.1 Hz, 2H), 4.63-4.44 (ABq, *J* = 14.7 Hz, Δν = 70.4 Hz, 2H), 4.40 (ABq, *J* = 10.3 Hz, Δν = 28.4 Hz, 2H), 4.23 (m, 1H), 3.86 (m, 2H), 3.82-2.70 (m, 3H), 3.80 (s, 3H), 3.68 (s, 3H), 3.62 (m, 2H), 3.54-3.38 (m, 2H), 3.34 (s, 3H), 3.12 (m, 1H), 2.66 (d, *J* = 4.41 Hz, 1H), 2.44-2.28 (m, 4H), 2.18 (m, 1H), 2.10-1.96 (m, 3H), 1.89-1.75 (m, 5H), 1.73-1.69 (m, 3H), 1.66-1.51 (m, 8H), 1.51-1.34 (m, 6H), 1.34-1.19 (m, 15H), 1.11 (m, 6H), 1.06 (s, 9H), 0.93 (s, 3H), 0.88 (t, *J* = 6.4 Hz, 3H), 0.84 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 172.3, 166.8, 159.3, 153.2, 144.4, 138.8, 137.8, 135.8, 134.1, 134.0, 131.4, 129.8, 129.5, 129.3, 129.2, 128.7, 128.0, 128.0, 127.9, 126.8, 114.0, 113.9, 108.9, 102.8, 94.0, 81.4, 81.4, 81.3, 79.2, 78.2, 75.9, 74.8, 73.4, 72.3, 72.0, 71.9, 71.2, 70.1, 68.1, 60.7, 55.5, 51.3, 51.2, 46.3, 42.8, 41.0, 39.9, 39.9, 39.8, 38.0, 36.9, 34.6, 32.5, 31.9, 29.9, 29.3, 29.3, 29.2, 27.6, 27.2, 27.1, 24.9, 24.2, 24.0, 22.8, 19.4, 17.0, 14.3; 125 MHz DEPT ($CDCl_3$)

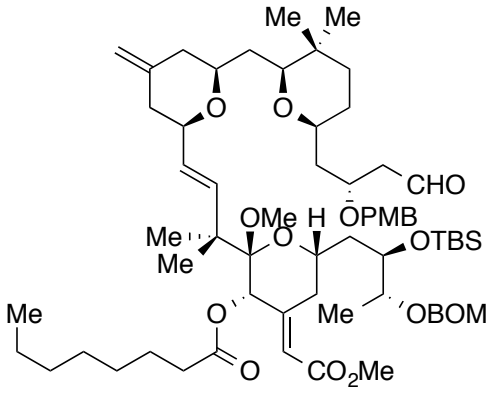
CH_3 δ 55.2, 51.1, 51.0, 27.4, 26.9, 26.9, 23.9, 23.8, 19.1, 16.8, 14.0; CH_2 δ 108.7, 93.8, 72.0, 71.8, 69.8, 60.4, 42.5, 40.8, 39.6, 39.6, 37.8, 36.7, 34.3, 31.6, 29.1, 29.0, 28.9, 24.7, 22.5; CH δ 138.6, 135.6, 129.5, 129.2, 129.0, 129.0, 128.5, 127.8, 127.6, 126.6, 113.8, 113.7, 81.1, 81.0, 79.0, 77.9, 77.9, 75.6, 74.6, 74.5, 73.2, 72.1, 71.6, 70.9, 69.9, 67.9; IR (neat) 3215, 3056, 2936, 2859, 1746, 1735, 1615, 1590, 1473, 1370, 1354, 1246, 1050, 824, 816 cm^{-1}; HRMS (EI) Calcd for $C_{76}H_{108}O_{14}SiNa$ ($M + Na^+$): 1295.7394. Found: 1295.7410.



Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,6*S*)-6-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((4-methoxybenzyl)oxy)butyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)tetrahydro-2*H*-pyran-3-yl octanoate 3.40. The alcohol **3.42** (37.7 mg, 0.029 mmol, 1 equiv) was dissolved in CH_2Cl_2 (1.5 mL, 0.02 M) in a 10 mL round-bottom flask and cooled to 0 °C, then 2,6-lutidine (42 μL, 0.354 mmol, 12 equiv) was added to the solution followed by TBSOTf (34 μL, 0.148 mmol, 5 equiv) dropwise via syringe. The reaction was stirred at 0 °C for 30 min and then quenched by the addition of MeOH (500 μL) followed by saturated $NaHCO_3$ solution (2 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5

mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification was accomplished by flash chromatography on a silica gel column (1 × 10 cm), eluting with 5% EtOAc/hexanes (800 mL), collecting 5 mL fractions. The fractions containing product (30-67) were combined and concentrated under reduced pressure to give the alcohol **3.40** (34.9 mg, 86%) as colorless oil: R_f = 0.61 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +16.5 (*c* = 0.150, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.70-7.64 (m, 4H), 7.45-7.30 (m, 11H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.94 (d, *J* = 16.1 Hz, 1H), 5.88 (s, 1H), 5.59 (s, 1H), 5.42 (dd, *J* = 16.1, 5.9 Hz, 1H), 4.79 (s, 2H), 4.79-4.43 (ABq, *J* = 18.6 Hz, Δν = 71.4 Hz, 2H), 4.63 (s, 2H), 4.40 (ABq, *J* = 10.3 Hz, Δν = 28.4 Hz, 2H), 4.09 (m, 2H), 3.90-3.70 (m, 3H), 3.80 (s, 3H), 3.68 (s, 3H), 3.60 (m, 1H), 3.54-3.43 (m, 2H), 3.30 (s, 3H), 3.12 (m, 1H), 2.34 (m, 3H), 2.16 (m, 1H), 2.04-1.95 (m, 2H), 1.87-1.75 (m, 4H), 1.69-1.51 (m, 6H), 1.49-1.34 (m, 4H), 1.33-1.22 (m, 12H), 1.16 (m, 5H), 1.10 (m, 6H), 1.06 (s, 9H), 0.92 (s, 3H), 0.87 (s, 9H), 0.83 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 167.6, 166.6, 159.3, 144.3, 138.3, 138.2, 135.8, 134.1, 134.1, 131.4, 129.8, 129.5, 129.5, 128.6, 128.0, 128.0, 127.9, 127.3, 114.0, 102.7, 93.3, 81.3, 79.1, 78.7, 75.9, 75.1, 74.7, 73.4, 72.3, 71.5, 70.4, 69.6, 68.5, 60.7, 55.5, 51.6, 51.3, 51.0, 46.1, 42.7, 41.6, 39.9, 38.8, 37.9, 36.9, 34.6, 32.5, 32.1, 31.9, 29.9, 29.3, 29.3, 27.6, 27.1, 26.1, 24.9, 24.2, 22.8, 19.4, 18.3, 14.3, 14.0, -3.8, -4.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.2, 50.9, 50.8, 50.8, 27.4, 26.9, 26.9, 25.9, 25.8, 19.1, 14.0, -4.5; CH_2 δ 108.9, 93.0, 72.0, 69.3, 60.4, 55.4, 42.5, 39.6, 37.8, 36.7, 34.2, 31.7, 31.6, 29.7, 29.2, 29.0, 24.6, 22.6; CH δ 135.6, 129.5, 129.2, 128.4, 127.8, 127.6, 113.8, 81.0, 78.5, 75.7, 74.5, 73.2; IR (neat) 3056, 2936, 2859, 1736, 17.45, 1615, 1590, 1473, 1370, 1354, 1146, 1050, 906, 824, 816

cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{76}H_{108}O_{14}SiNa$ (M + Na^+): 1409.8265. Found: 1409.8275.

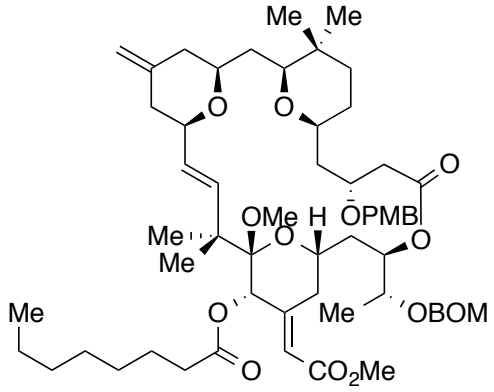


Preparation of (2*S*,3*S*,6*S*,*E*)-6-((2*R*,3*R*)-3-((benzyloxy)methoxy)-2-((*tert*-butyldimethylsilyl)oxy)butyl)-2-methoxy-4-(2-methoxy-2-oxoethylidene)-2-((*E*)-4-((2*R*,6*S*)-6-(((2*S*,6*S*)-6-((*R*)-2-((4-methoxybenzyl)oxy)-4-oxobutyl)-3,3-dimethyltetrahydro-2*H*-pyran-2-yl)methyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-2-methylbut-3-en-2-yl)tetrahydro-2*H*-pyran-3-yl octanoate 3.43. The BPS ether **3.40** (31.3 mg, 0.023 mmol, 1 equiv) was dissolved in DMF (2.3 mL, 0.01 M) in a 10 mL round-bottom flask at rt. To this solution were added TBAF (46 μL, 1 M in THF, 0.046 mmol, 2 equiv) and a solution of AcOH (46 μL, 1 M in DMF, 0.046 mmol, 2 equiv). The solution was stirred for 2 d and then diluted with 50% EtOAc/hexanes (2 mL) and quenched with water (2 mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This material was then taken to the next step without further purification.

To a stirring solution of alcohol (assumed to be 0.023 mmol, 1.0 equiv) in CH_2Cl_2 (250 μL, 0.1 M), in a 5 mL reaction vial at 0 °C, were added diisopropylethylamine (31 μL, 0.176 mmol, 7.0 equiv) and DMSO (18 μL, 0.252 mmol, 10.0 equiv). The solution

was stirred at -10 °C for 5 min and SO_3·Py (16 mg, 0.101 mmol, 4.0 equiv) was added in one portion. Stirring continued at -10 °C for 1.5 h, after which the reaction mixture was diluted with CH_2Cl_2 (1 mL) and quenched by the addition of saturated aqueous $NaHCO_3$ solution (1 mL). The mixture was stirred at rt for 10 min until effervescence was complete. The reaction mixture was then partitioned between CH_2Cl_2 (5 mL) and saturated aqueous $NaHCO_3$ solution (5 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography on a silica gel column (1 × 10 cm), eluting with 10% EtOAc/hexanes, collecting 9 mL fractions. The fractions containing product (45-53) were combined and concentrated under reduced pressure to provide the aldehyde **3.43** (20 mg, 78%) as colorless oil: R_f = 0.51 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +16.5 (*c* = 0.500, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 9.79 (t, *J* = 2 Hz, 1H), 7.38-7.26 (m, 7H), 7.25 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 5.96 (d, *J* = 16.1 Hz, 1H), 5.89 (s, 1H), 5.59 (s, 1H), 5.43 (dd, *J* = 15.6, 5.9 Hz, 1H), 4.80 (s, 2H), 4.64 (m, 3H), 4.48 (m, 3H), 4.17 (m, 1H), 4.10 (m, 2H), 3.89-3.78 (m, 1H), 3.81 (s, 3H), 3.75 (m, 1H), 3.68 (s, 3H), 3.56 (m, 1H), 3.53-3.42 (m, 3H), 3.31 (s, 3H), 3.11 (m, 1H), 2.62 (m, 2H), 2.36 (m, 4H), 2.19 (d, *J* = 13.7 Hz, 1H), 2.01 (m, 2H), 1.89-1.70 (m, 5H), 1.69-1.54 (m, 8H), 1.53-1.35 (m, 6H), 1.35-1.15 (m, 8H), 0.93-0.86 (m, 15H), 0.83 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 201.7, 172.5, 167.6, 166.7, 159.6, 144.3, 138.6, 138.1, 137.5, 130.6, 129.5, 129.4, 128.6, 128.6, 128.0, 127.9, 127.8, 127.1, 126.9, 114.1, 109.3, 102.7, 93.3, 93.2, 81.4, 79.4, 76.0, 75.1, 74.8, 72.4, 72.1, 70.3, 69.6, 66.0, 55.5, 51.6, 51.3, 51.0, 49.6, 46.1, 42.6, 40.9, 39.9, 39.7, 36.9, 34.6, 32.5, 31.9, 29.9, 29.5, 29.2, 27.6,

26.0, 25.6, 24.9, 24.2, 23.6, 22.8, 19.3, 18.2, 15.5, 14.3, 13.9, -3.8, -4.2, -4.4; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.3, 50.8, 27.3, 25.8, 19.0, 14.0, -4.5; CH_2 δ 109.0, 93.0, 72.1, 69.3, 49.3, 42.3, 39.6, 39.4, 36.7, 34.3, 31.7, 29.7, 29.0, 29.0, 24.6, 22.6; CH δ 201.4, 129.3, 129.1, 128.4, 127.8, 127.6, 113.9, 81.2, 78.8, 77.2, 75.8, 74.8, 74.5, 71.9; IR (neat) 3059, 2936, 2859, 1737, 1612, 1473, 1368, 1355, 1243, 1050, 903, 820, 801 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{66}H_{102}O_{14}SiNa$ ($M + Na^+$): 1169.6931. Found: 1169.6945.



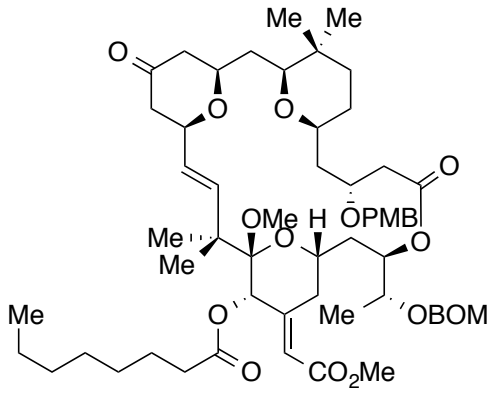
Preparation of (1*S*,3*S*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10,26,26-tetramethyl-5-methylene-19-oxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 3.44. To a stirring solution of the aldehyde **3.43** (24.5 mg, 0.022 mmol, 1.0 equiv) in 2-methyl-2-butene (548 μL, 0.04 M) and *tert*-butyl alcohol (1 mL), in a 5 mL reaction vial at rt, was added aqueous KH_2PO_4 solution (438 μL, 1 M in H_2O). The mixture was cooled to -10 °C, and $NaClO_2$ (39.6 mg, 0.438 mmol, 20.0 equiv) was added in one portion. The reaction mixture stirred vigorously at -10 °C for 4 h, and was then quenched by the addition of aqueous pH 4 buffer solution (1 mL). The reaction mixture was partitioned between CH_2Cl_2 (5 mL) and aqueous pH 4 buffer solution (5 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic

phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was taken directly to the next step without further purification.

To a stirring solution of TBS ether (assumed to be 0.022 mmol) in 9:1 THF/pyridine (2.1 mL, 0.01 M) in a 5 mL plastic vial were added methanol (210 μL) and HF·Py (20 %, 1.1 mL). The solution was stirred at rt for 2 d, and then diluted with 50% EtOAc/hexanes (100 mL), and washed with brine (2 × 10 mL). The solution was dried over Na_2SO_4 and concentrated under reduced pressure. The crude seco-acid was taken on to the next step without purification.

To a stirring solution of seco-acid (assumed 0.022 mmol) in THF (756 μL, 0.03 M) at 0 °C in a 15 mL round-bottom flask were added triethylamine (19 μL, 0.136 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (680 μL, 0.1 M in THF, 0.068 mmol, 3.0 equiv) by syringe. The solution was stirred at 0 °C for 5 min, then warmed to rt and stirred for 2 h. The reaction mixture was diluted with 1:3 THF/toluene (9 mL, 0.0025 M), and taken up into a 10.0 mL gas-tight syringe. The resulting solution was added into a stirring solution of 4-dimethylaminopyridine (55 mg, 0.454 mmol, 20 equiv) in toluene (15 mL, 0.0015 M) in a 50 mL round-bottom flask at 40 °C over 12 h by means of a syringe pump. The vial was rinsed with toluene (2 mL) and the rinsing solution was added into the reaction mixture by syringe pump over 3 h. The solution was cooled to rt and diluted with 50 mL of 40% EtOAc/hexanes. The solution was washed with saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash chromatography on a 1.5 × 10 cm silica gel column, eluting with 5% EtOAc/hexanes, collecting 5 mL fractions. The fractions containing product

(13-17) were combined and concentrated under reduced pressure to give the product macrolactone **3.44** (12 mg, 50%) as a colorless oil: R_f = 0.64 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +12.5 (*c* = 0.250, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.44-7.26 (m, 5H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.23 (d, *J* = 15.6 Hz, 1H), 5.94 (m, 1H), 5.58 (ddd, *J* = 11.7, 3.9, 1.9 Hz, 1H), 5.33 (dd, *J* = 8.8, 6.8 Hz, 1H), 5.16 (s, 1H), 4.81 (m, 4H), 4.64 (ABq, *J* = 12.2 Hz, Δν = 21.0 Hz, 2H), 4.51-4.40 (ABq, *J* = 10.3 Hz, Δν = 29.8 Hz, 2H), 4.11 (m, 1H), 3.99-3.91 (m, 2H), 3.77-3.65 (m, 2H), 3.74 (s, 3H), 3.69 (s, 3H), 3.43 (dd, *J* = 9.8, 9.3 Hz, 1H), 3.31 (m, 1H), 3.08 (s, 3H), 2.78 (d, *J* = 10.3 Hz, 1H), 2.63 (dd, *J* = 15.2, 2.5 Hz, 1 H), 2.46-2.18 (m, 6H), 2.16-1.95 (m, 4H), 1.95-1.76 (m, 4H), 1.75-1.51 (m, 6H), 1.49-1.19 (m, 10H), 1.09 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.82 (s, 3H), 0.79 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 172.4, 172.2, 166.9, 159.3, 151.5, 144.6, 142.6, 138.2, 134.5, 131.1, 129.6, 128.6, 128.1, 127.8, 125.5, 119.5, 113.9, 108.9, 103.4, 93.7, 82.9, 81.7, 73.9, 73.0, 71.9, 70.5, 69.8, 62.3, 55.5, 52.9, 51.3, 45.2, 43.3, 41.6, 41.2, 40.8, 39.1, 38.8, 34.8, 34.4, 32.2, 31.8, 31.1, 29.9, 29.2, 29.0, 27.3, 26.2, 24.9, 22.8, 20.0, 18.9, 14.9, 14.2; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.2, 52.6, 51.0, 27.0, 26.0, 19.8, 18.7, 14.7, 14.0; CH_2 δ 108.7, 93.5, 71.7, 69.5, 43.0, 41.3, 40.9, 40.6, 38.8, 38.6, 34.6, 34.1, 31.6, 30.8, 29.7, 29.0, 28.9, 28.8, 24.6, 22.5; CH δ 142.3, 129.3, 128.4, 127.8, 127.6, 125.3, 119.2, 113.6, 82.7, 81.4, 76.7, 76.5, 76.4, 73.6, 72.7, 70.3, 67.0; IR (neat) 3046, 2936, 2859, 1737, 1615, 1590, 1473, 1370, 1354, 1246, 1050, 907, 824, 816 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{60}H_{86}O_{14}Na$ ($M + Na^+$): 1053.591. Found: 1053.5915.



Preparation of (1*S*,3*R*,7*R*,8*E*,11*S*,12*S*,13*E*,15*S*,17*R*,21*R*,23*S*)-17-((*R*)-1-((benzyloxy)methoxy)ethyl)-11-methoxy-13-(2-methoxy-2-oxoethylidene)-21-((4-methoxybenzyl)oxy)-10,10,26,26-tetramethyl-5,19-dioxo-18,27,28,29-tetraoxatetracyclo[21.3.1.1^{3,7}.1^{11,15}]nonacos-8-en-12-yl octanoate 3.45. A 100 ml flask containing 50 mL of CH_2Cl_2 was cooled to -78 °C, and a stream of O_3 was passed in for 5 min. The color of the solution changed to light blue. The flask was sealed and kept at -78 °C for immediate use. This O_3 solution was added in 100 μL portion via a plastic syringe to a stirring solution of olefin **3.44** (8.4 mg, 0.008 mmol, 1.0 equiv) in CH_2Cl_2 (1 mL, 0.01 M) at -78 °C. The reaction was monitored by TLC and the addition of the O_3 solution was continued every 10 min until the starting material was fully consumed. Dimethyl sulfide (2 mL) was then added and the mixture was warmed to rt. The solution was stirred at rt for 12 h, after which the solvent was removed under reduced pressure. Purification was accomplished using flash chromatography on a silica gel column (1 × 10 cm), eluting with 15% EtOAc/hexanes (600 mL), collecting 5 mL fractions. The fractions containing product (38-58) were combined and concentrated under reduced pressure to provide ketone **3.45** (6 mg, 78%) as colorless oil: R_f = 0.50 (30% EtOAc/hexanes); $[\alpha]^{20}_D$ = +25.5 (*c* = 0.200, $CHCl_3$); 500 MHz ^{1}H NMR ($CDCl_3$) δ 7.39-7.28 (m, 5H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.30 (d, *J* = 15.6 Hz, 1H), 5.95 (m, 1H), 5.58 (m,

1H), 5.32 (dd, J = 15.6, 8.8 Hz, 1H), 5.17 (s, 1H), 4.80 (ABq, J = 6.8 Hz, Δν = 17.6 Hz, 2H), 4.64 (ABq, J = 11.7 Hz, Δν = 20.0 Hz, 2H), 4.49-4.34 (ABq, J = 10.8 Hz, Δν = 53.0 Hz, 2H), 4.28 (ddd, J = 8.8, 8.8, 3.9 Hz, 1H), 4.05 (m, 1H), 3.91 (m, 1H), 3.81-3.62 (m, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.30 (m, 1H), 3.08 (s, 3H), 2.72 (m, 1H), 2.57 (m, 2H), 2.45-2.34 (m, 4H), 2.28 (m, 2H), 2.20-1.96 (m, 5H), 1.85 (m, 3H), 1.67-1.49 (m, 4H), 1.49-1.10 (m, 14H), 1.01 (m, 4H), 0.88 (t, J = 6.4 Hz, 3H), 0.83 (s, 3H), 0.77 (s, 3H); 125 MHZ ^{13}C NMR ($CDCl_3$) δ 206.8, 172.4, 171.9, 166.7, 159.1, 151.1, 143.5, 137.9, 130.5, 129.4, 128.4, 127.8, 127.6, 123.8, 119.3, 113.7, 103.0, 93.5, 82.0, 79.7, 75.1, 73.5, 72.8, 71.4, 70.3, 69.5, 67.0, 55.2, 52.5, 51.1, 48.4, 47.7, 45.0, 40.6, 38.7, 34.6, 34.1, 31.8, 31.6, 30.8, 29.7, 28.9, 28.8, 26.9, 25.9, 24.6, 22.5, 19.6, 18.6, 14.7, 14.0; 125 MHz DEPT ($CDCl_3$) CH_3 δ 55.2, 52.5, 26.9, 25.9, 18.6, 14.7, 14.0; CH_2 δ 93.5, 71.4, 69.5, 53.5, 47.7, 43.2, 40.7, 38.7, 34.6, 34.1, 31.6, 30.8, 29.7, 28.9, 28.8, 24.6; CH δ 143.6, 129.4, 128.4, 127.8, 127.6, 123.8, 119.3, 113.7, 82.0, 79.7, 77.2, 76.5, 76.8, 75.1, 73.5, 72.8, 70.3, 67.0, 28.6, 17.9; IR (neat) 2936, 2859, 2347, 1732, 1652, 1590, 1473, 1370, 1354, 1246, 1050, 824, 716 cm^{-1}; HRMS (ESI/TOF) Calcd for $C_{59}H_{84}O_{15}Na$ ($M + Na^+$): 1055.5702. Found: 1055.5714.

References

1. Kazanietz, M. G.; Lewin, N. E.; Gao, F.; Pettit, G. R.; Blumberg, P. M., *Mol. Pharmacol.* **1994**, *46*, 374-379.

2. For the latest information on clinical trials with bryostatin 1, please see. http://clinicaltrials.gov.

3. (a) Spitaler, M.; Utz, I.; Hilbe, W.; Hofmann, J.; Grunicke, H. H., *Biochem. Pharmacol.* **1998**, *56*, 861-869; (b) Koutcher, J. A.; Motwani, M.; Zakian, K. L.; Li, X.-K.; Matei, C.; Dyke, J. P.; Ballon, D.; Yoo, H.-H.; Schwartz, G. K., *Clin. Cancer Res.* **2000**, *6*, 1498; (c) Basu, A.; Lazo, J. S., *Cancer Res.* **1992**, *52*, 3119-3124; (d)

Mohammad, R. M.; Wall, N. R.; Dutcher, J. A.; Katib, A. M., *Clin. Cancer Res.* **2000**, *6*, 4950-4956; (e) Berkow, R. L.; Kraft, A. S., *Biochem. Biophys. Res. Commun.* **1985**, *131*, 1109-1116; (f) Bosco, M. C.; Rottschafer, S.; Taylor, L. S.; Ortaldo, J. R.; Longo, D. L.; Espinoza-Delgado, I., *Blood* **1997**, *89*, 3402-3411; (g) Sun, M.-K.; Alkon, D. L., *Eur. J. Pharmacol.* **2005**, *512*, 43-51; (h) Kuzirian, A. M.; Epstein, H. T.; Gagliardi, C. J.; Nelson, T. J.; Sakakibara, M.; Taylor, C. G.; Scioletti, A. B.; Alkon, D. L., *Biological Bulletin* **2006**, *210*, 201-214; (i) Sun, M.-K.; Alkon, D. L., *Eur. J. Pharmacol.* **2008**, *584*, 328-337; (j) http://www.brni.org; (k) Mehla, R.; Bivalkar-Mehla, S.; Zhang, R.; Handy, I.; Albrecht, H.; Giri, S.; Nagarkatti, P.; Nagarkatti, M.; Chauhan, A., *PLoS ONE* **2010**, *5*, e11160.

4. (a) Keck, G. E.; Poudel, Y. B.; Rudra, A.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4084-4088; (b) Kedei, N.; Lubart, E. S.; Lewin, N. E.; Telek, A.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Keck, G. E.; Kolusheva, S.; Jelinek, R.; Blumberg, P. M., *ChemBioChem* **2011**, *12*, 1242-1251; (c) Kedei, N.; Telek, A.; Czap, A.; Lubart, E. S.; Czifra, G.; Yang, D.; Chen, J.; Morrison, T.; Goldsmith, P. K.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Li, W.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2011**, *81*, 1296-1308; (d) Kedei, N.; Telek, A.; Michalowski, A. M.; Kraft, M. B.; Li, W.; Poudel, Y. B.; Rudra, A.; Petersen, M. E.; Keck, G. E.; Blumberg, P. M., *Biochem. Pharmacol.* **2013**, *85*, 313-324; (e) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *J. Am. Chem. Soc.* **2008**, *130*, 6660-6661; (f) Keck, G. E.; Li, W.; Kraft, M. B.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 2277-2280; (g) Keck, G. E.; Poudel, Y. B.; Welch, D. S.; Kraft, M. B.; Truong, A. P.; Stephens, J. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M., *Org. Lett.* **2009**, *11*, 593-596.

5. (a) Barton, D. H. R.; McCombie, S. W., *J. Chem. Soc., Perkin Trans.* **1975**, 1574-1585; (b) Barton, D. H. R.; Jaszberenyi, J. C., *Tetrahedron Lett.* **1989**, *30*, 2619-2622.

6. (a) Williams, D. R.; Meyer, K. G.; Shamim, K.; Patnaik, S., *Can. J. Chem.* **2004**, *82*, 120-130; (b) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T., *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12058-12063; (c) Corey, E. J.; Imwinkelried, R.; Pikul, S.; Xiang, Y. B., *J. Am. Chem. Soc.* **1989**, *111*, 5493-5495; (d) Corey, E. J.; Yu, C.-M.; Kim, S. S., *J. Am. Chem. Soc.* **1989**, *111*, 5495-5496; (e) Williams, D. R.; Brooks, D. A.; Meyer, K. G.; Clark, M. P., *Tetrahedron Lett.* **1998**, *39*, 7251-7254.

7. Voight, E. A.; Seradj, H.; Roethle, P. A.; Burke, S. D., *Org. Lett.* **2004**, *6*, 4045-4048.

8. Aurell, M. J.; Gil, S.; Mestres, R.; Parra, M.; Parra, L., *Tetrahedron* **1998**, *54*, 4357-4366.

9. Keck, G. E.; Andrus, M. B.; Romer, D. R., *J. Org. Chem.* **1991**, *56*, 417-420.

10. Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I., *Acc. Chem. Res.* **1998**, *31*, 9-17.

11. (a) Mitsunobu, O.; Yamada, Y., *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380-2382; (b) Mitsunobu, O., *Synthesis* **1981**, 1-28.

12. Ghosh, A. K.; Cappiello, J.; Dongwoo, S., *Tetrahedron Lett.* **1998**, *39*, 4651-4654.

13. Knight, D. W.; Morgan, I. R.; Proctor, A. J., *Tetrahedron Lett.* **2010**, *51*, 638-640.

14. (a) Schaufelberger, D. E.; Chmurny, G. N.; Koleck, M. P., *Magn. Reson. Chem.* **1991**, *29*, 366-374; (b) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J., *J. Am. Chem. Soc.* **1982**, *104*, 6846-6848.

15. (a) Seco, J. M.; Quinoa, E.; Riguera, R., *Chem. Rev.* **2004**, *104*, 17-117; (b) Dale, J. A.; Mosher, H. S., *J. Am. Chem. Soc.* **1968**, *90*, 3732.

16. Li, W. Dissertation Thesis, University of Utah, 2011.

17. (a) Parikh, J. R.; Doering, W. V. E., *J. Am. Chem. Soc.* **1967**, *89*, 5505-5507; (b) Lindgren, B. O.; Nilsson, T., *Acta Chem. Scand.* **1973**, *27*, 888-890.

18. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M., *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993.

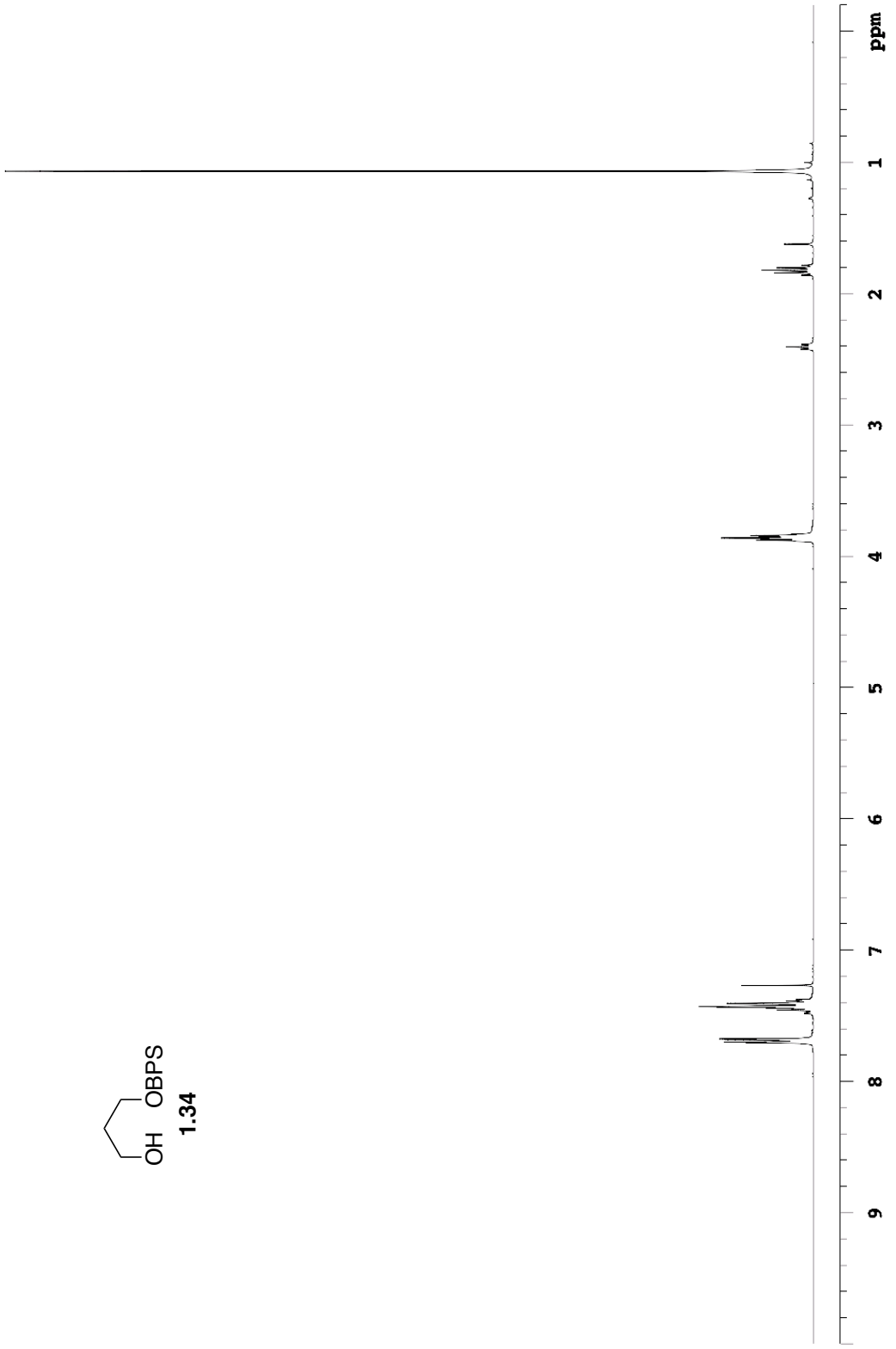
19. Tanaka, K.; Otsubo, K.; Fuji, K., *Tetrahedron Lett.* **1996**, *37*, 3735-3738.

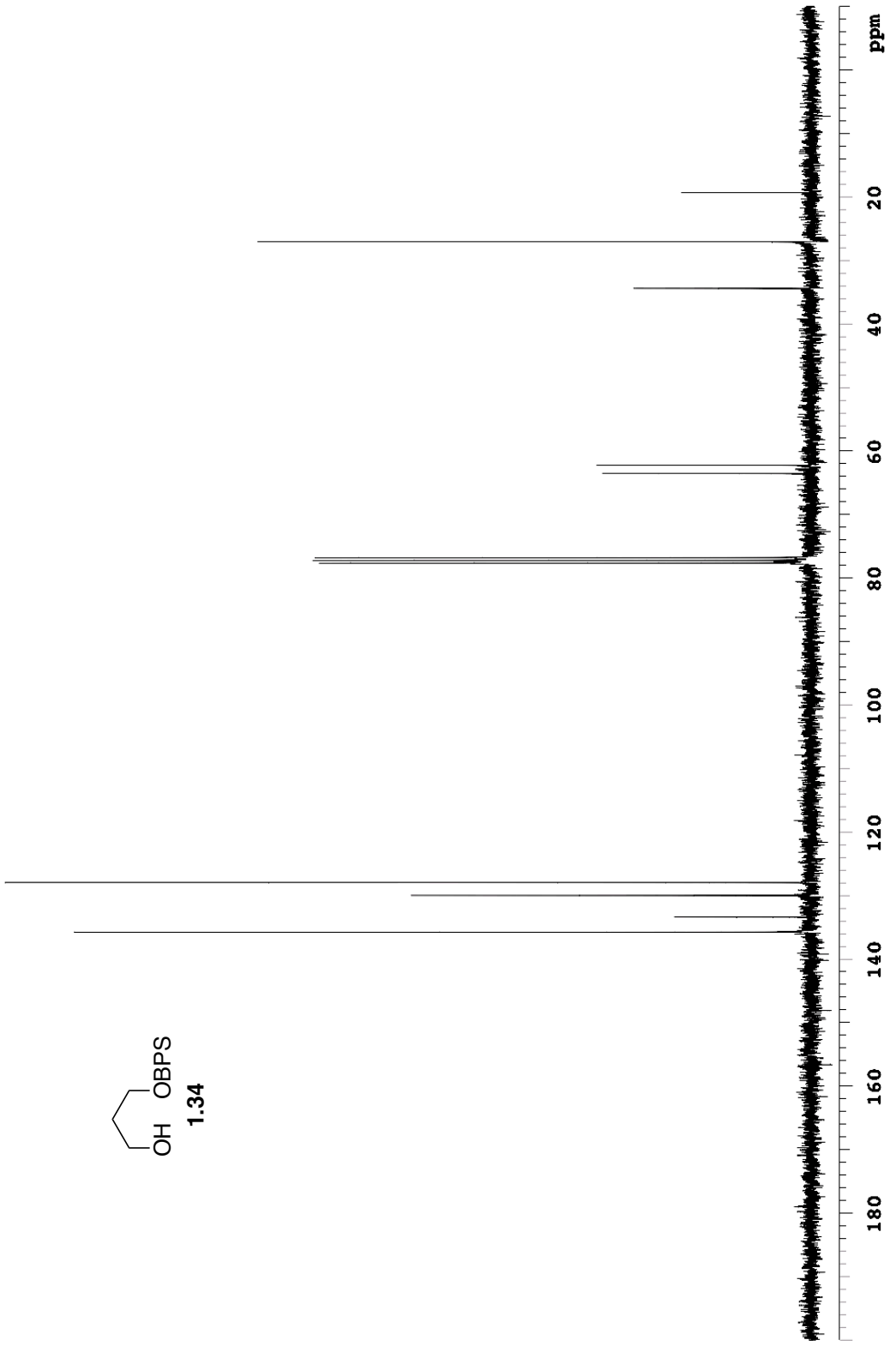
20. Armarego, W. L. H.; Perrin, D. D., *Purification of Laboratory Chemicals*. 4 ed.; Butterworth-Heinemann: Oxford: 1997.

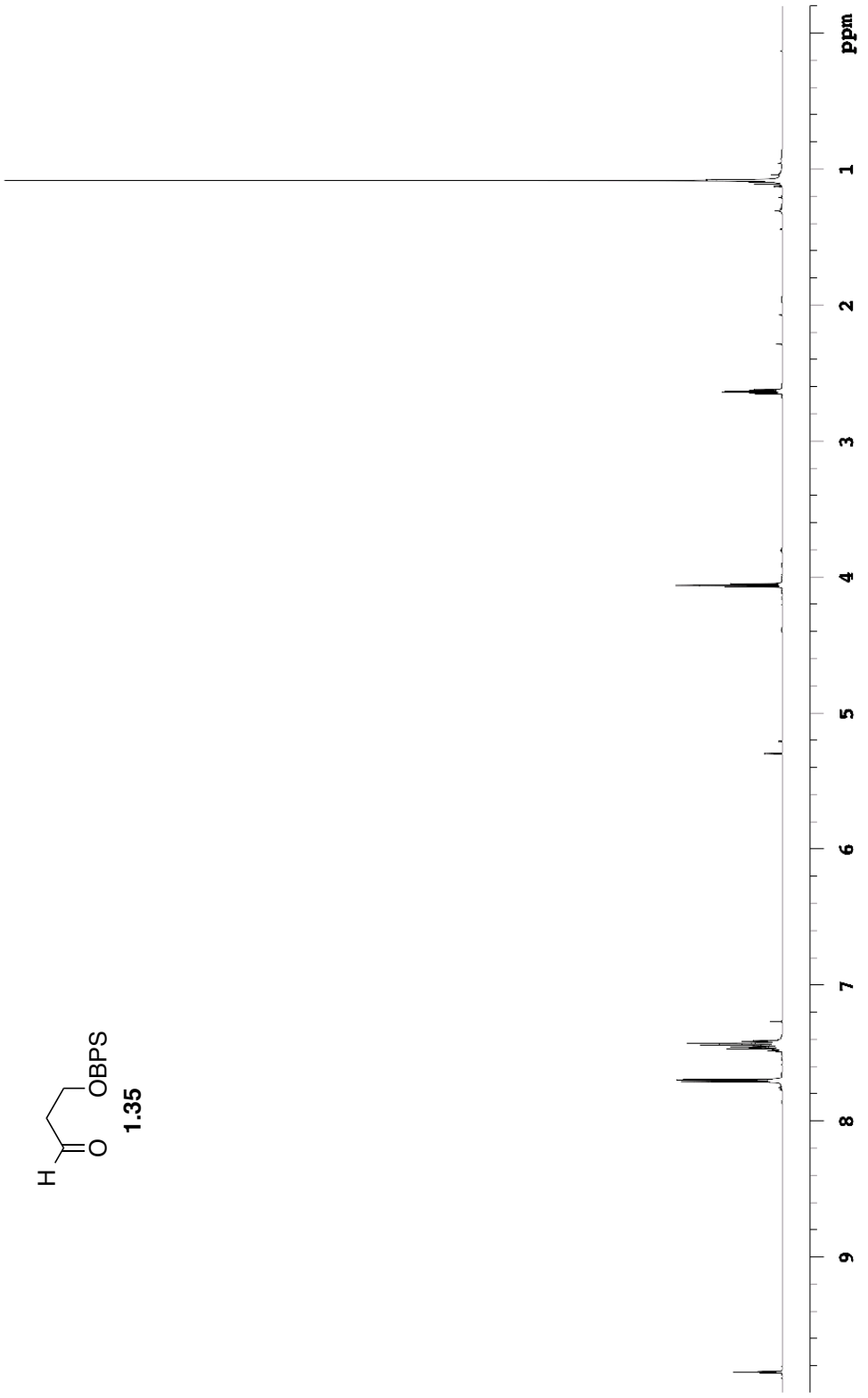
21. Watson, S. C.; Eastham, J. F., *J. Organomet. Chem.* **1967**, *9*, 165-168.

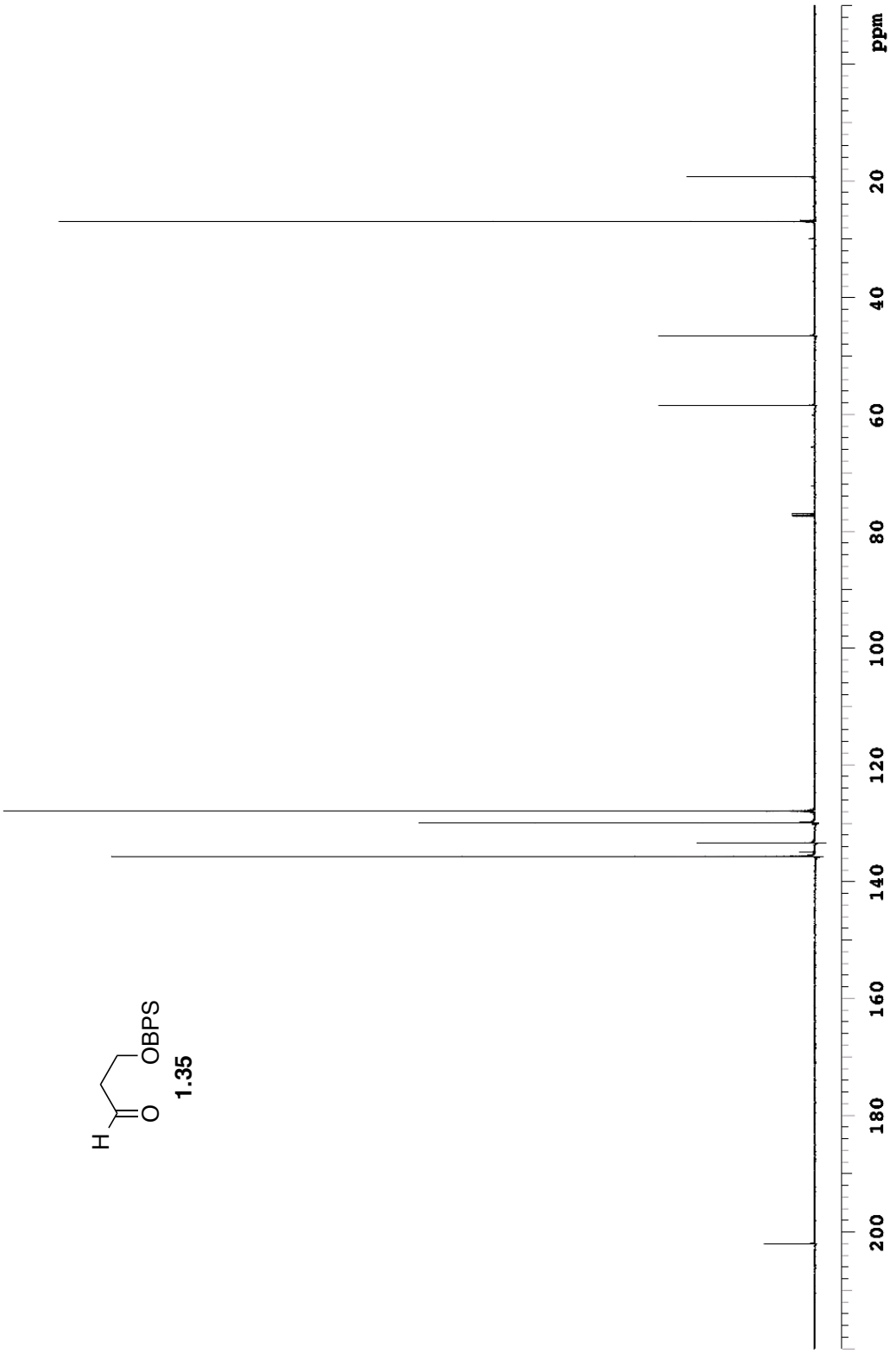
APPENDIX A

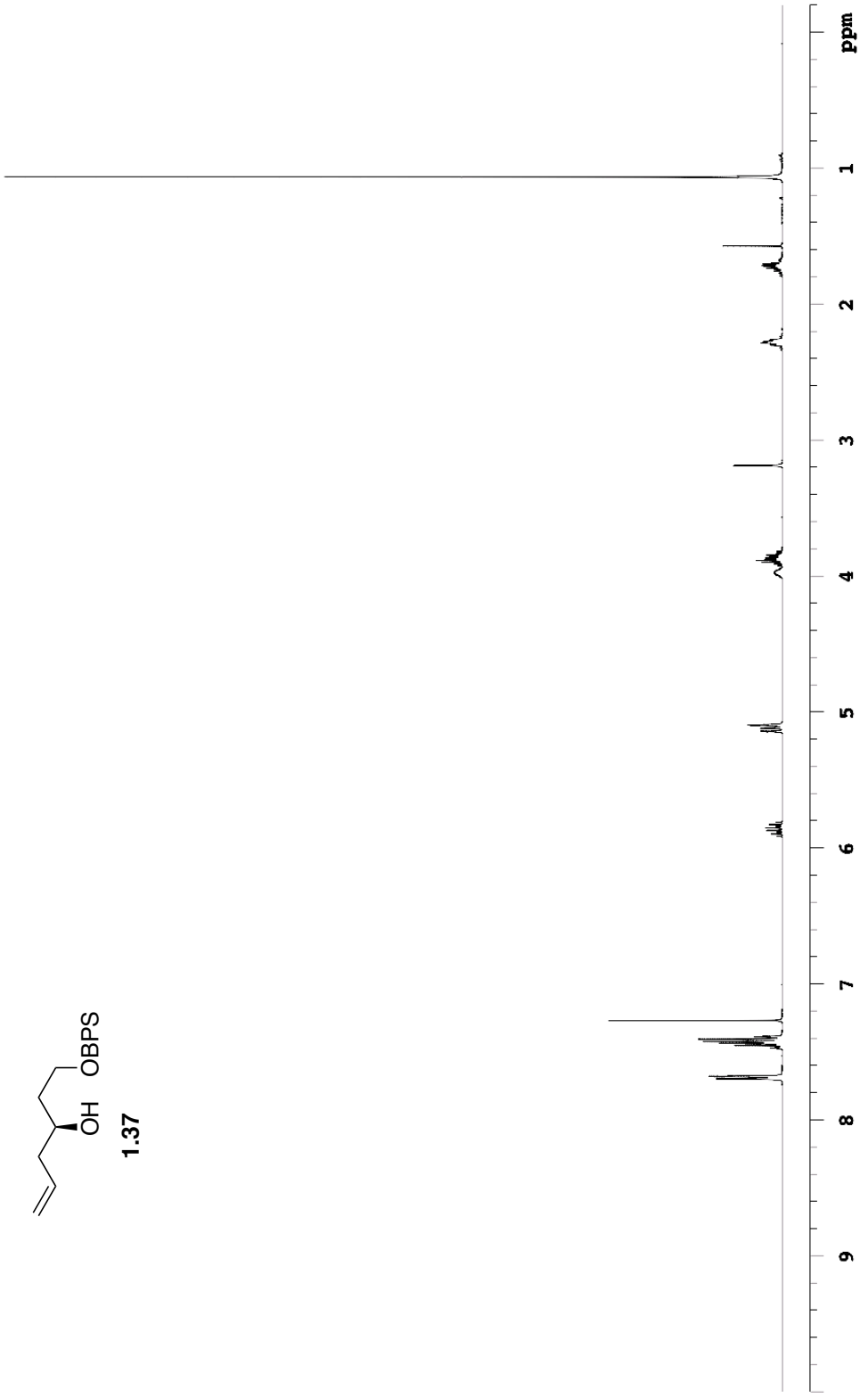
^{1}H, AND ^{13}C NMR SPECTRA FOR CHAPTER 1

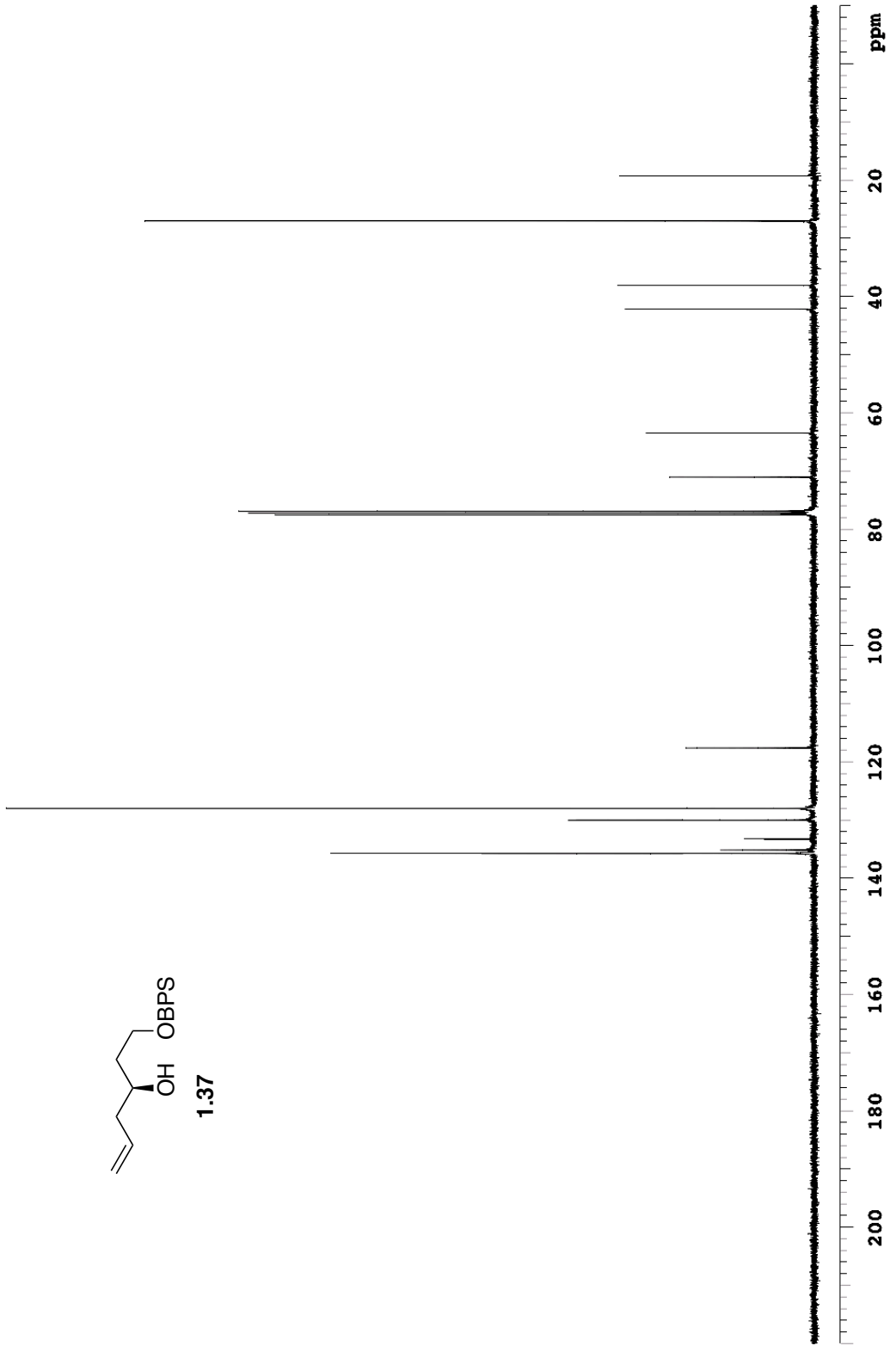

OH OBPS
1.34
ppm
1
2
3
4
5
6
7
8
9

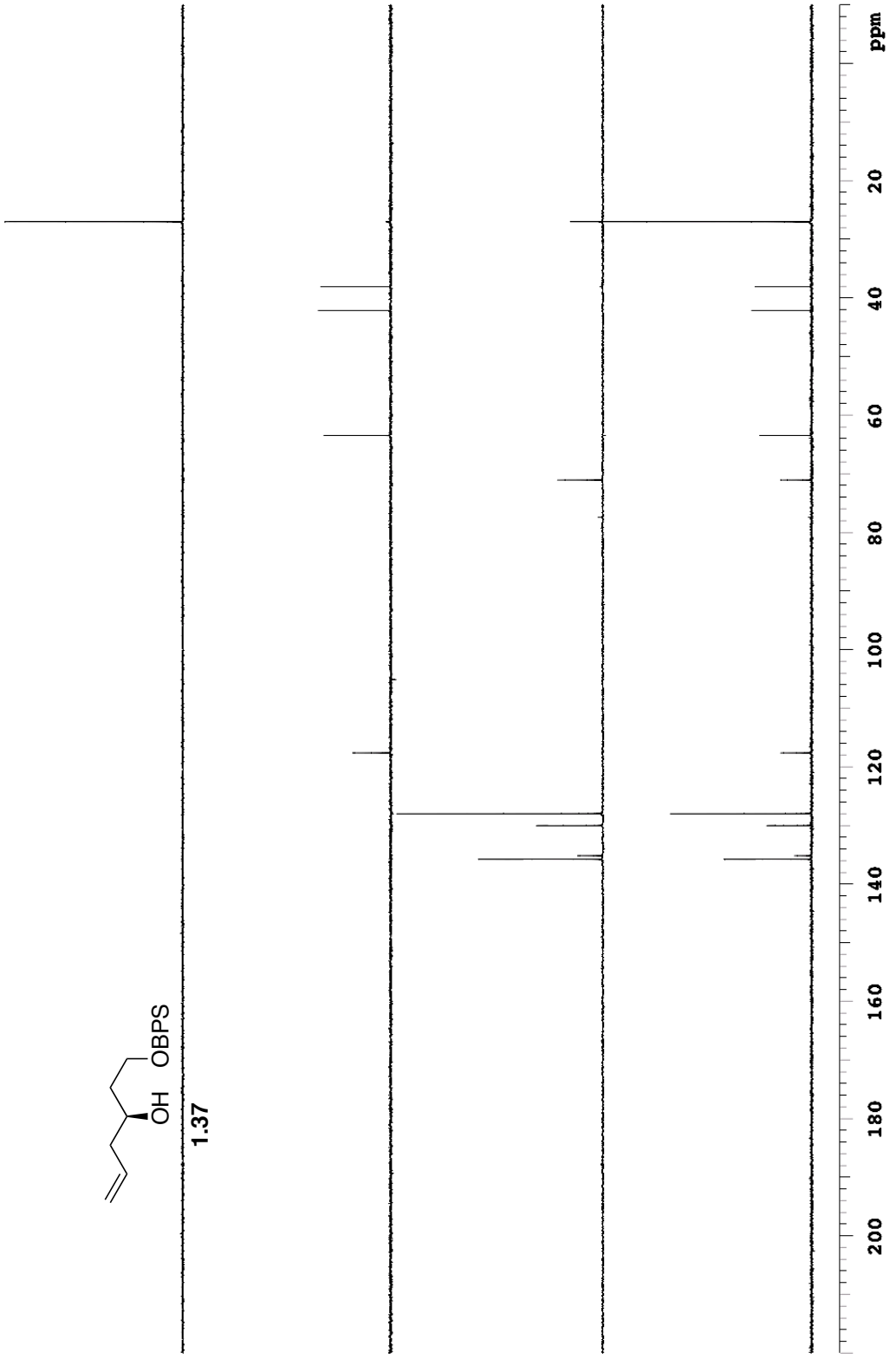

OH OBPS
1.34
ppm
20
40
60
80
100
120
140
160
180

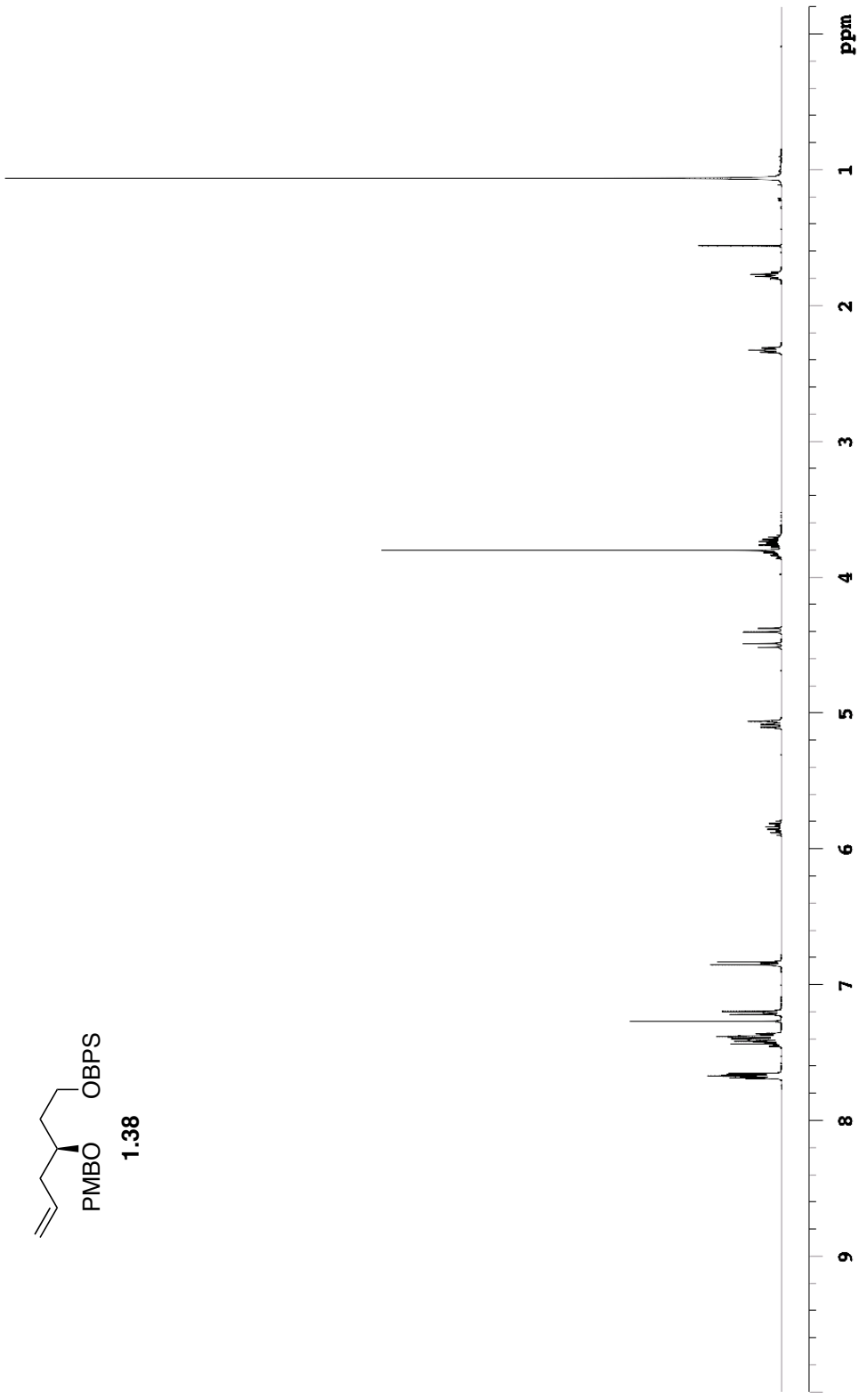

H
O
OBPS
1.35
ppm
1
2
3
4
5
6
7
8
9

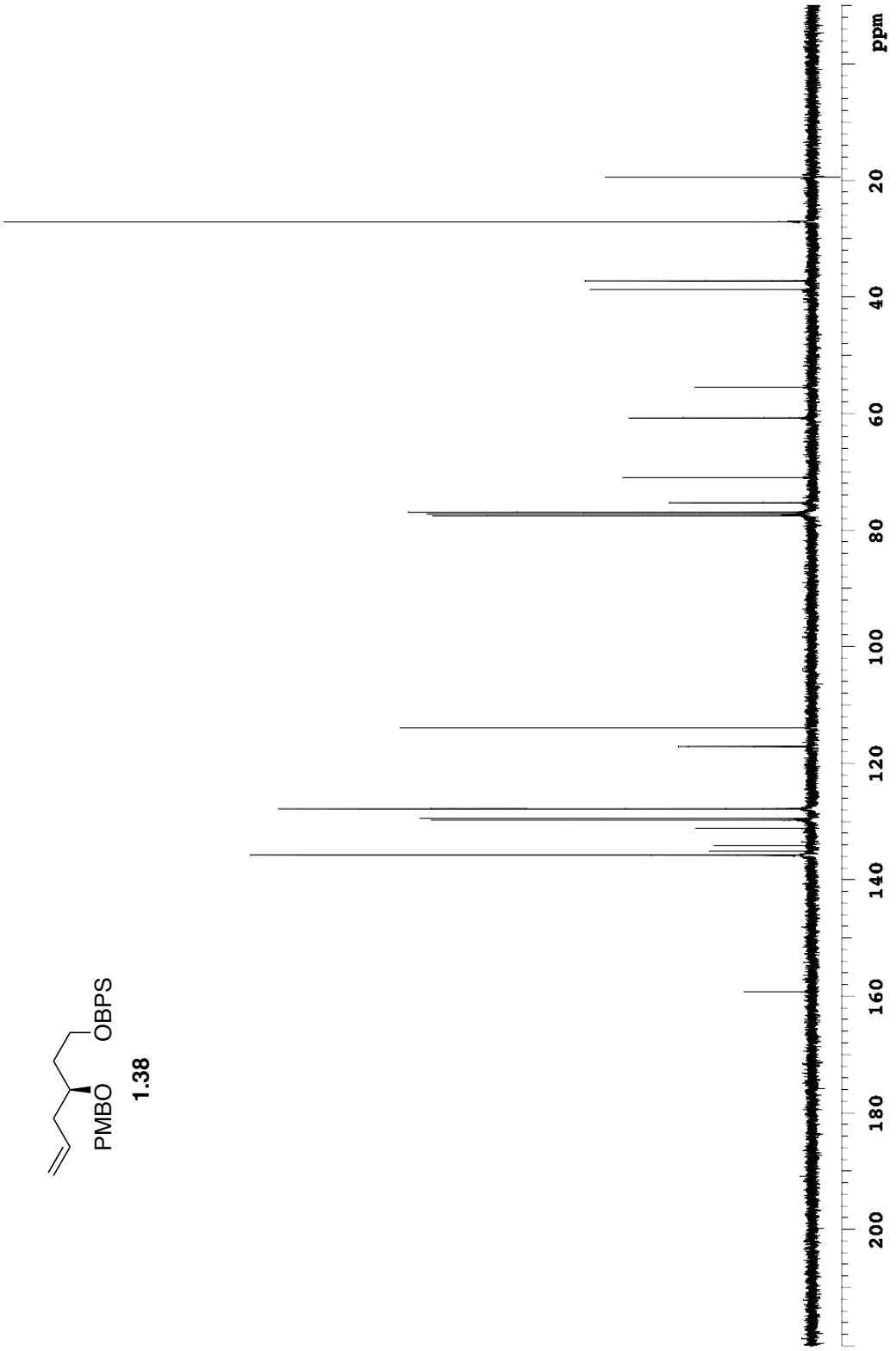

OBPS
O
H
1.35
ppm
20
40
60
80
100
120
140
160
180
200


OBPS
OH
1.37
ppm
1
2
3
4
5
6
7
8
9

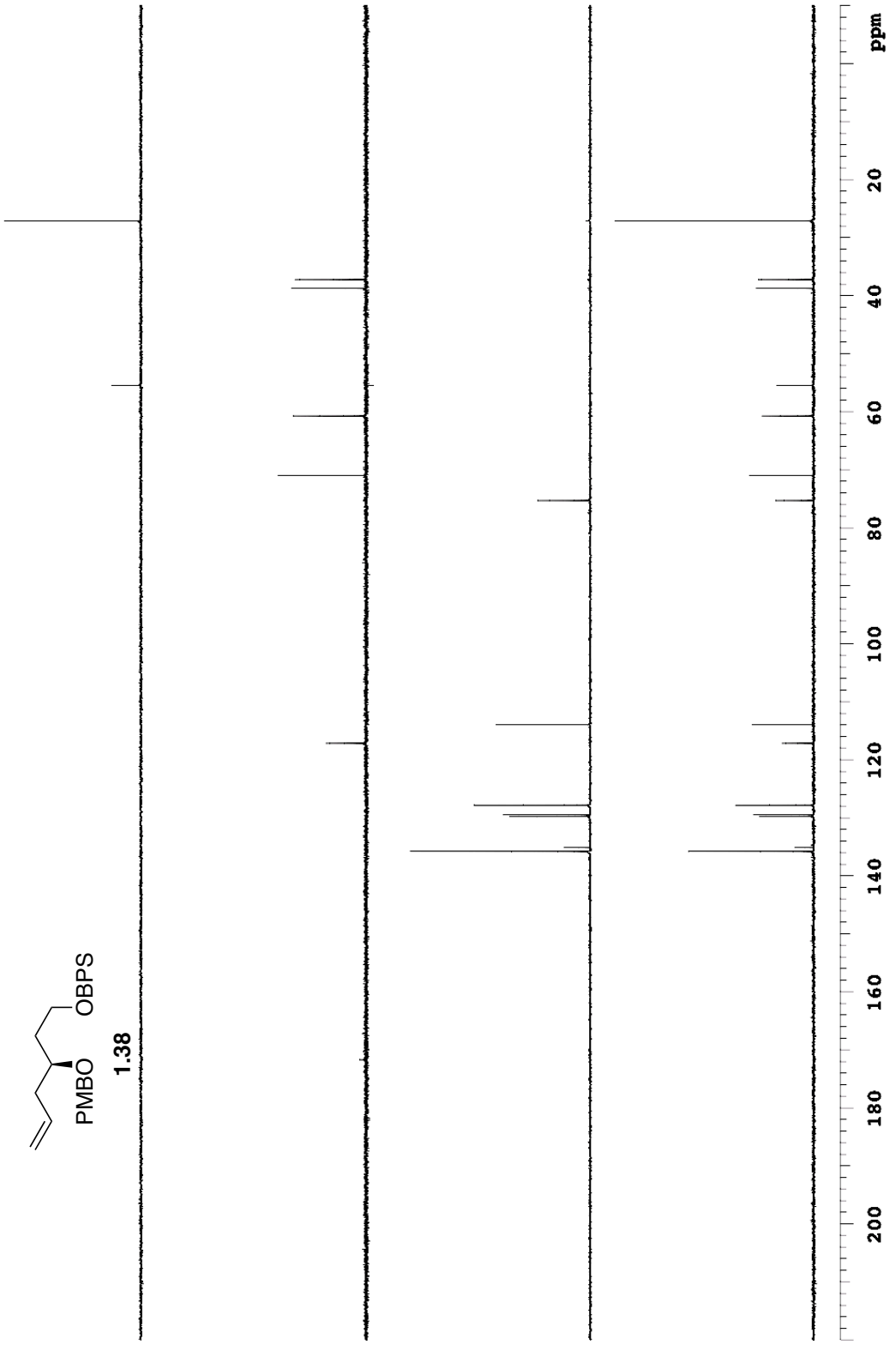

OBPS
OH
1.37
ppm
20
40
60
80
100
120
140
160
180
200

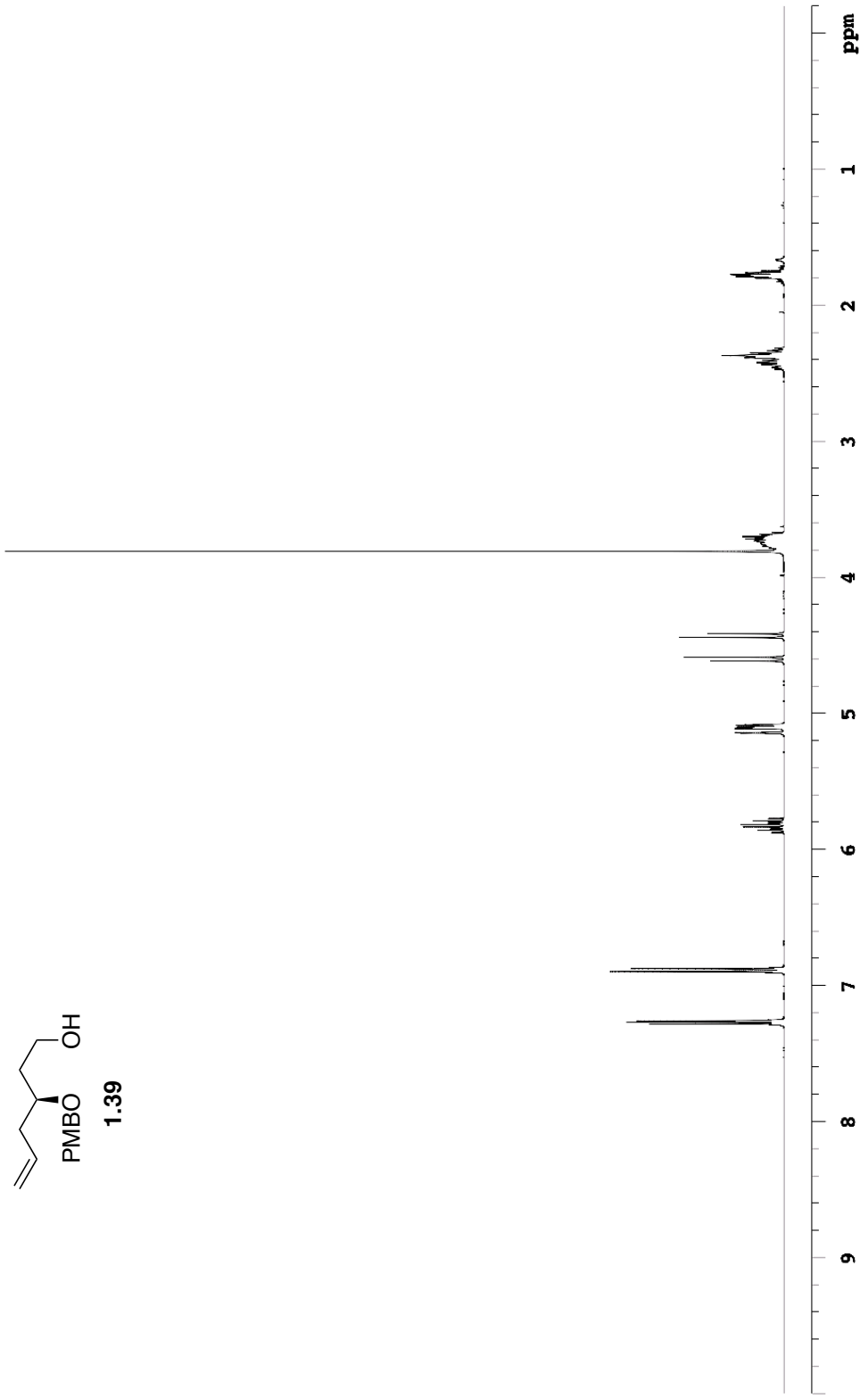

OH OBPS
1.37
ppm
20
40
60
80
100
120
140
160
180
200

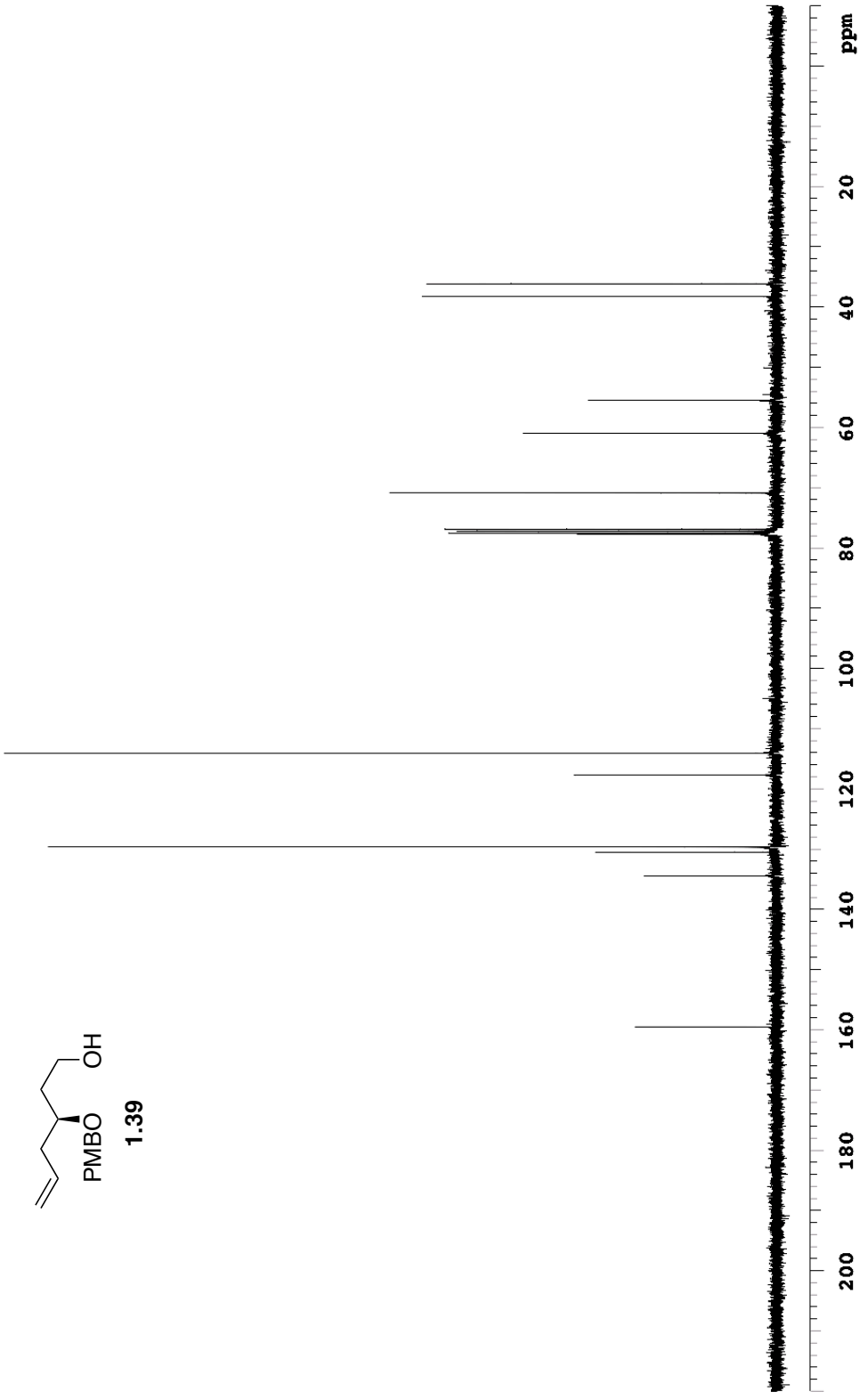

PMBO
OBPS
1.38
ppm
1
2
3
4
5
6
7
8
9

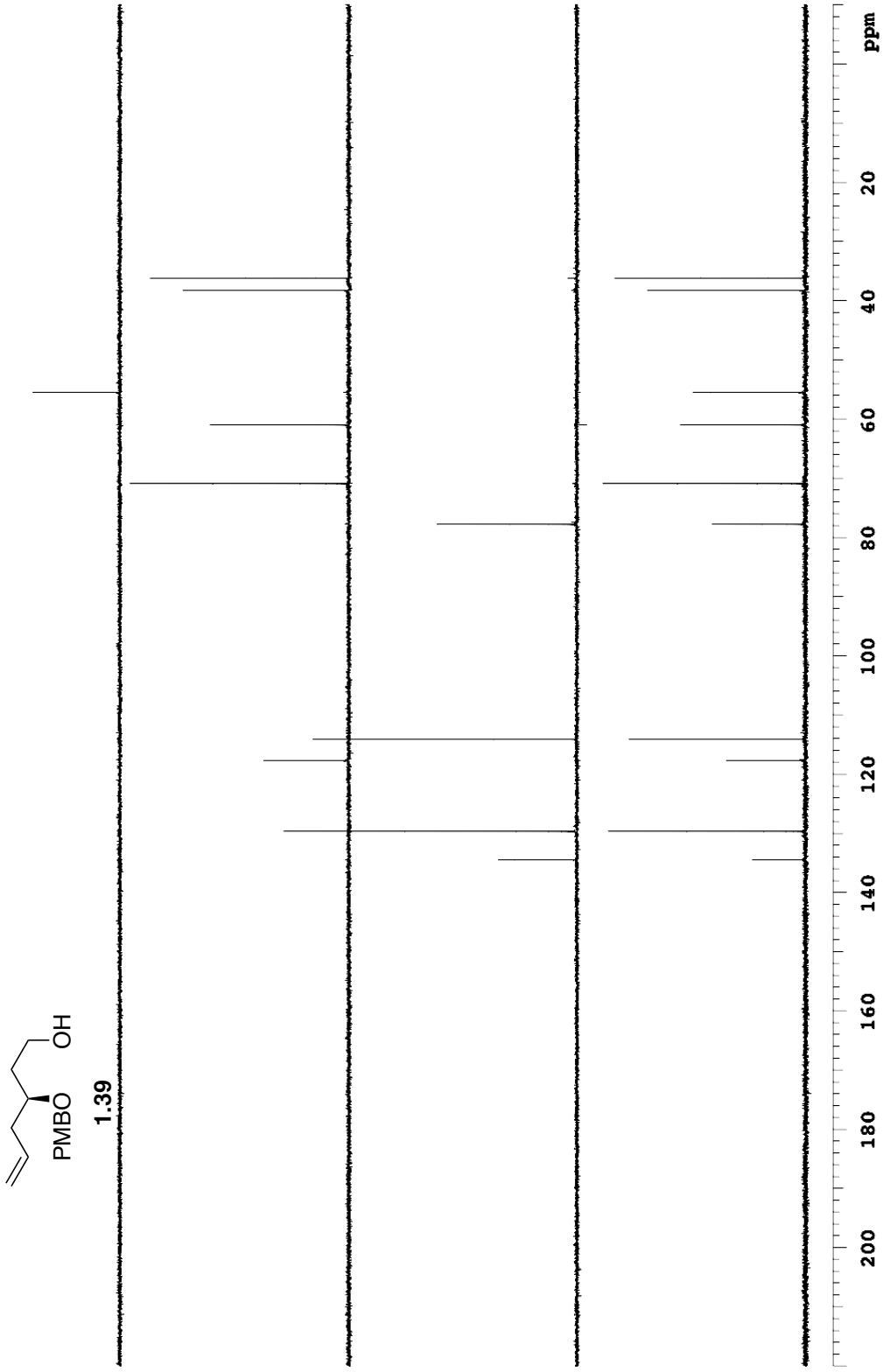


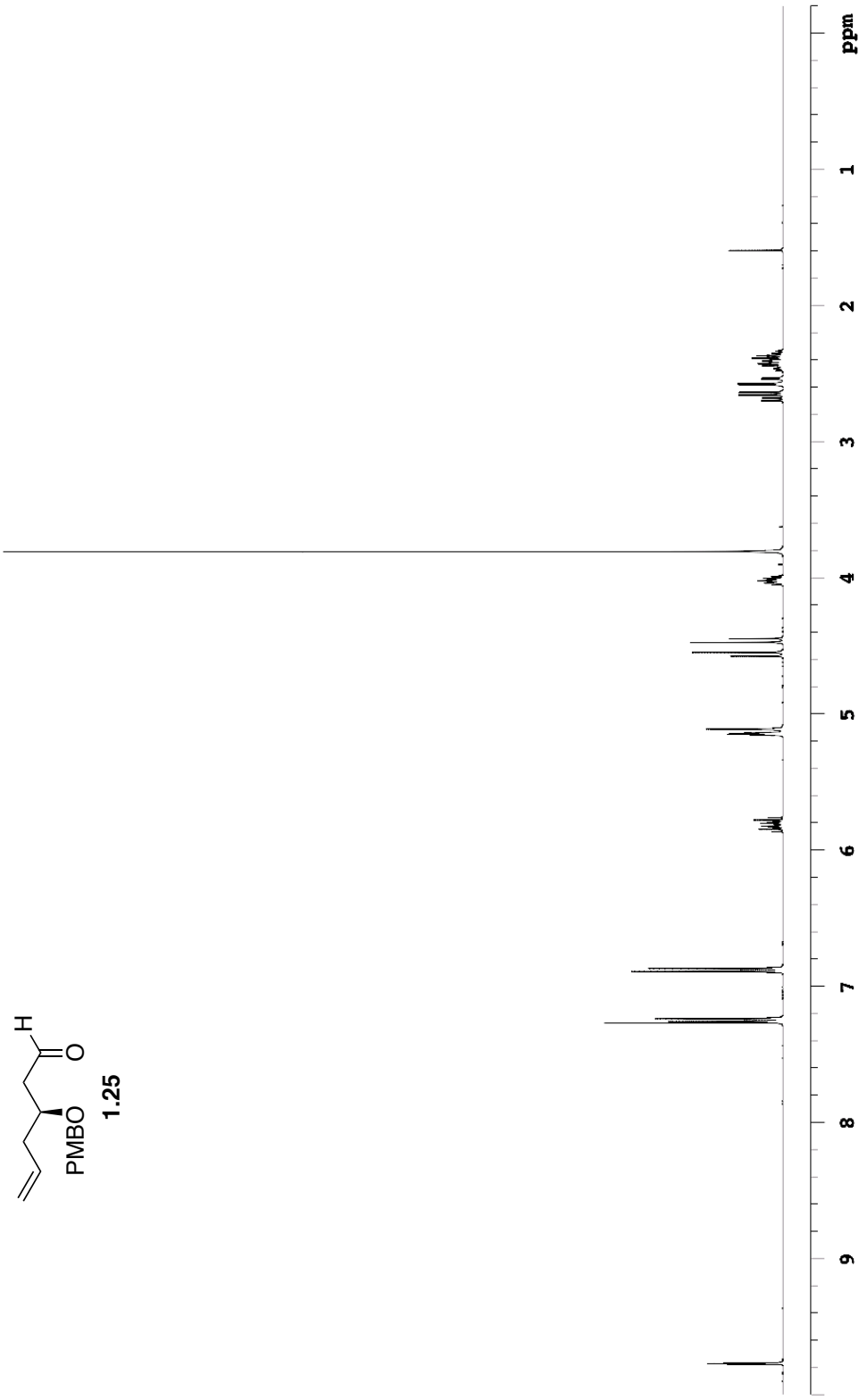
1.38

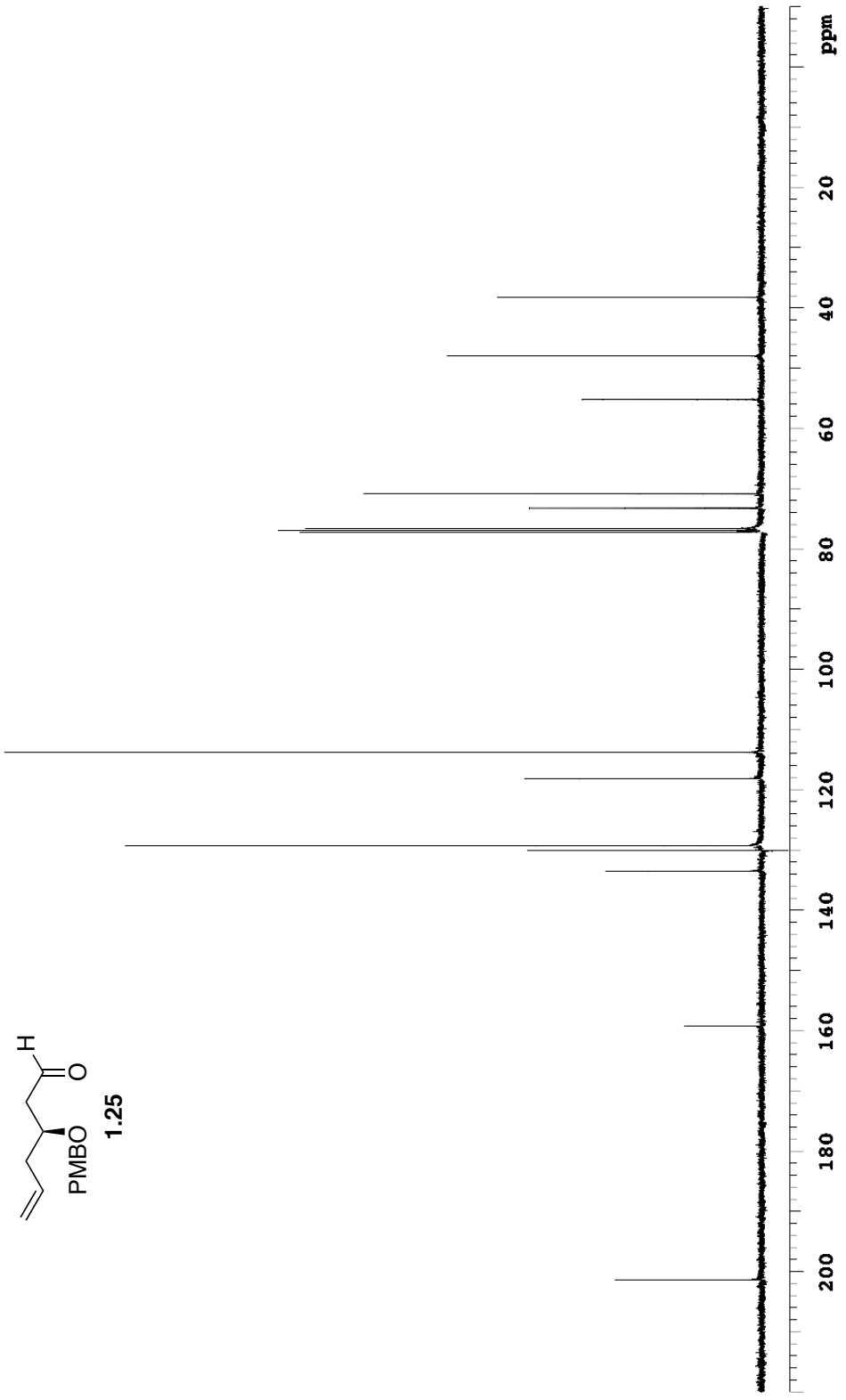

PMBO
OBPS
1.38
ppm
200
180
160
140
120
100
80
60
40
20

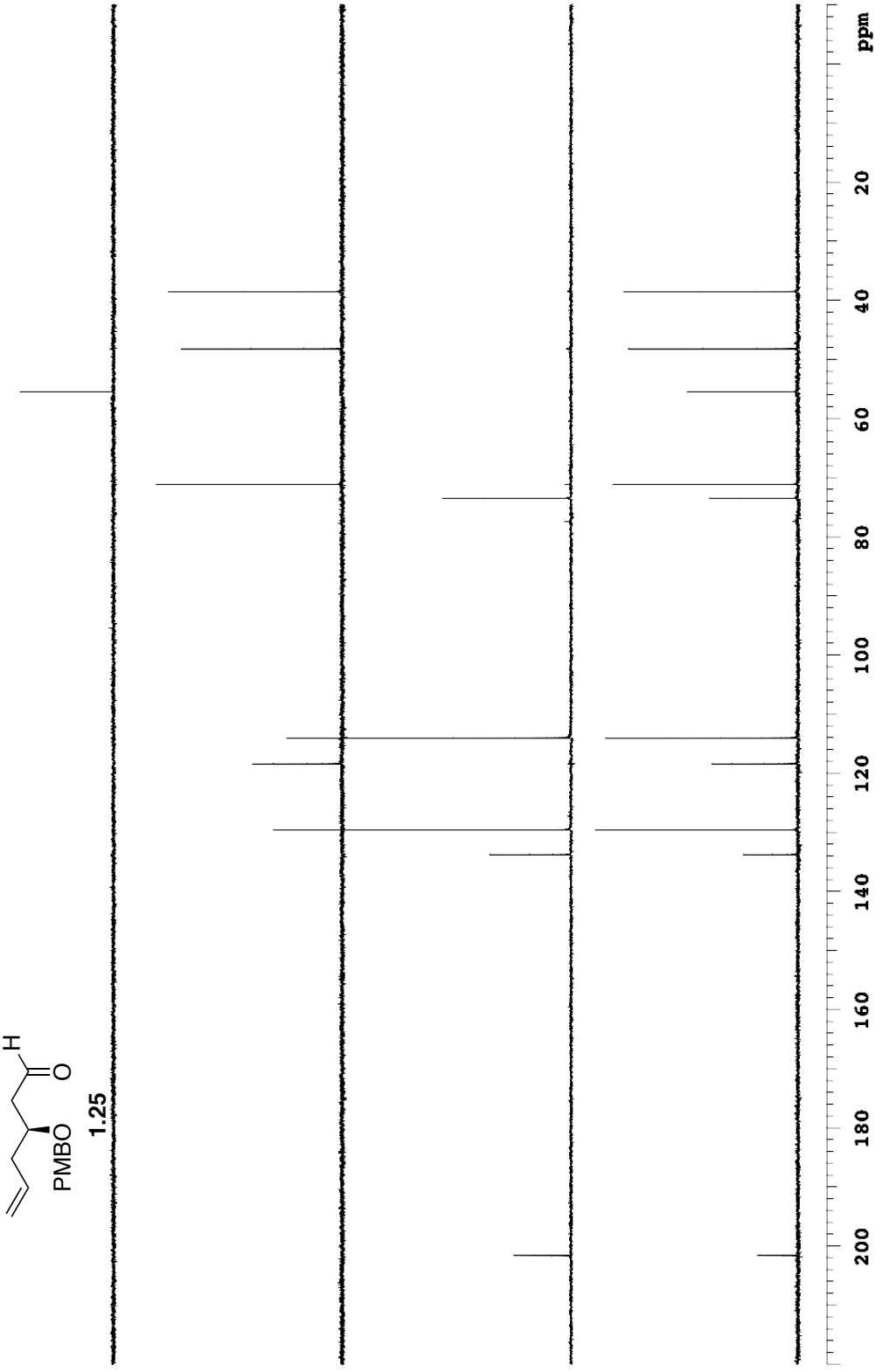

PMBO
OH
1.39
ppm
1
2
3
4
5
6
7
8
9

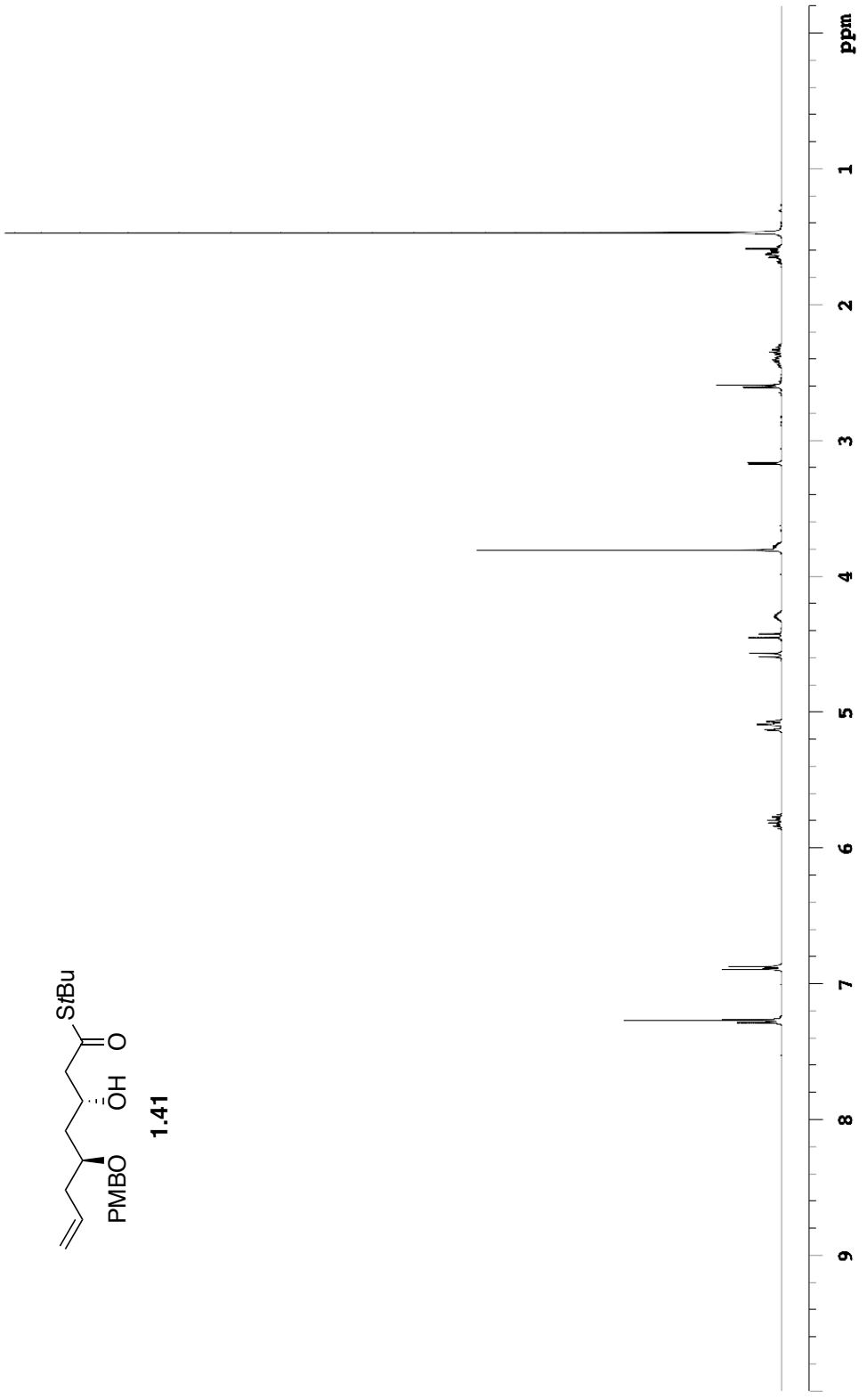

PMBO
OH
1.39
ppm
200
180
160
140
120
100
80
60
40
20


PMBO
OH
1.39
ppm
200
180
160
140
120
100
80
60
40
20

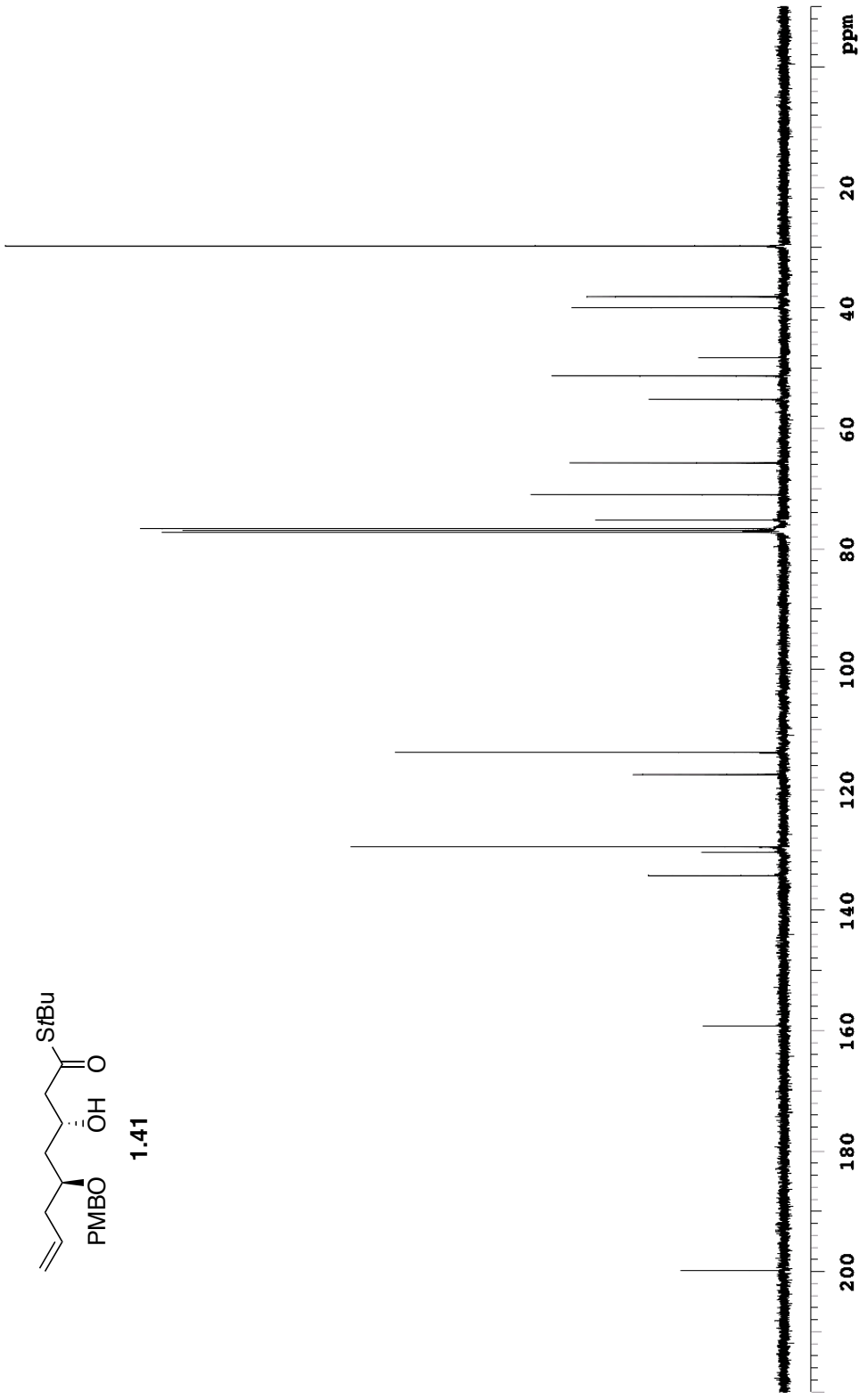

H
O
PMBO
1.25
ppm
1
2
3
4
5
6
7
8
9


H
O
PMBO
1.25
ppm
20
40
60
80
100
120
140
160
180
200

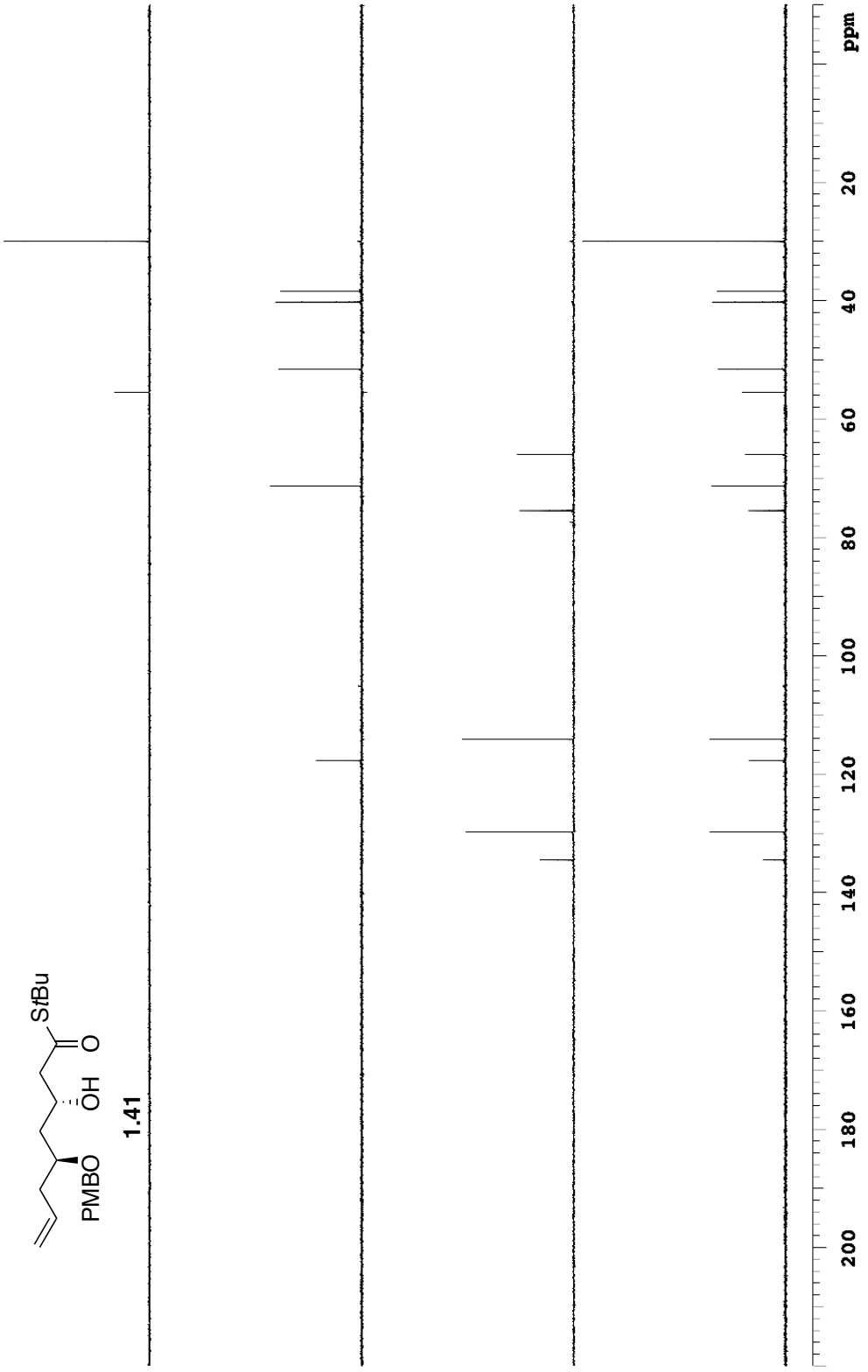

H
O
PMBO
1.25
ppm
200
180
160
140
120
100
80
60
40
20

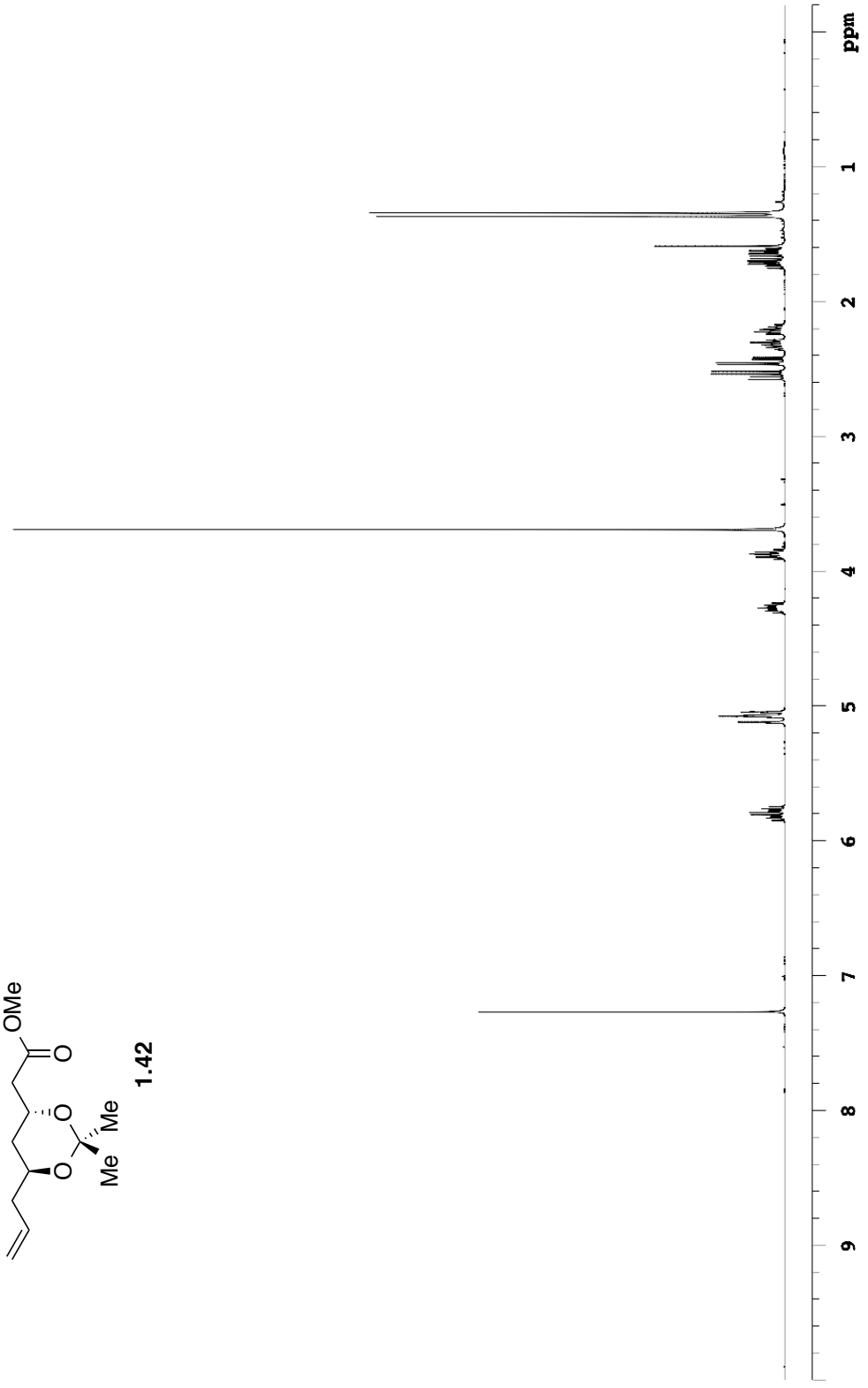


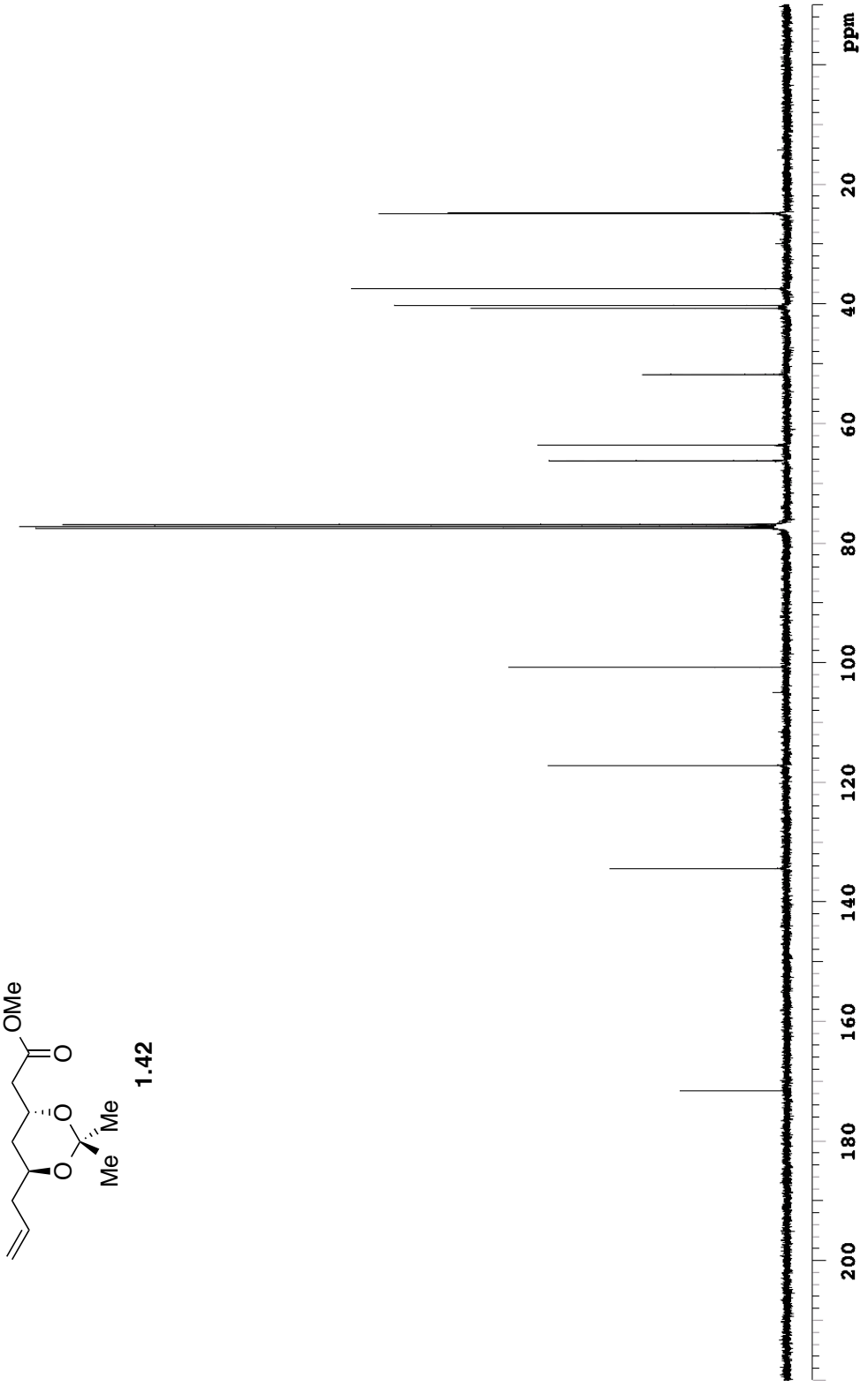
1.41

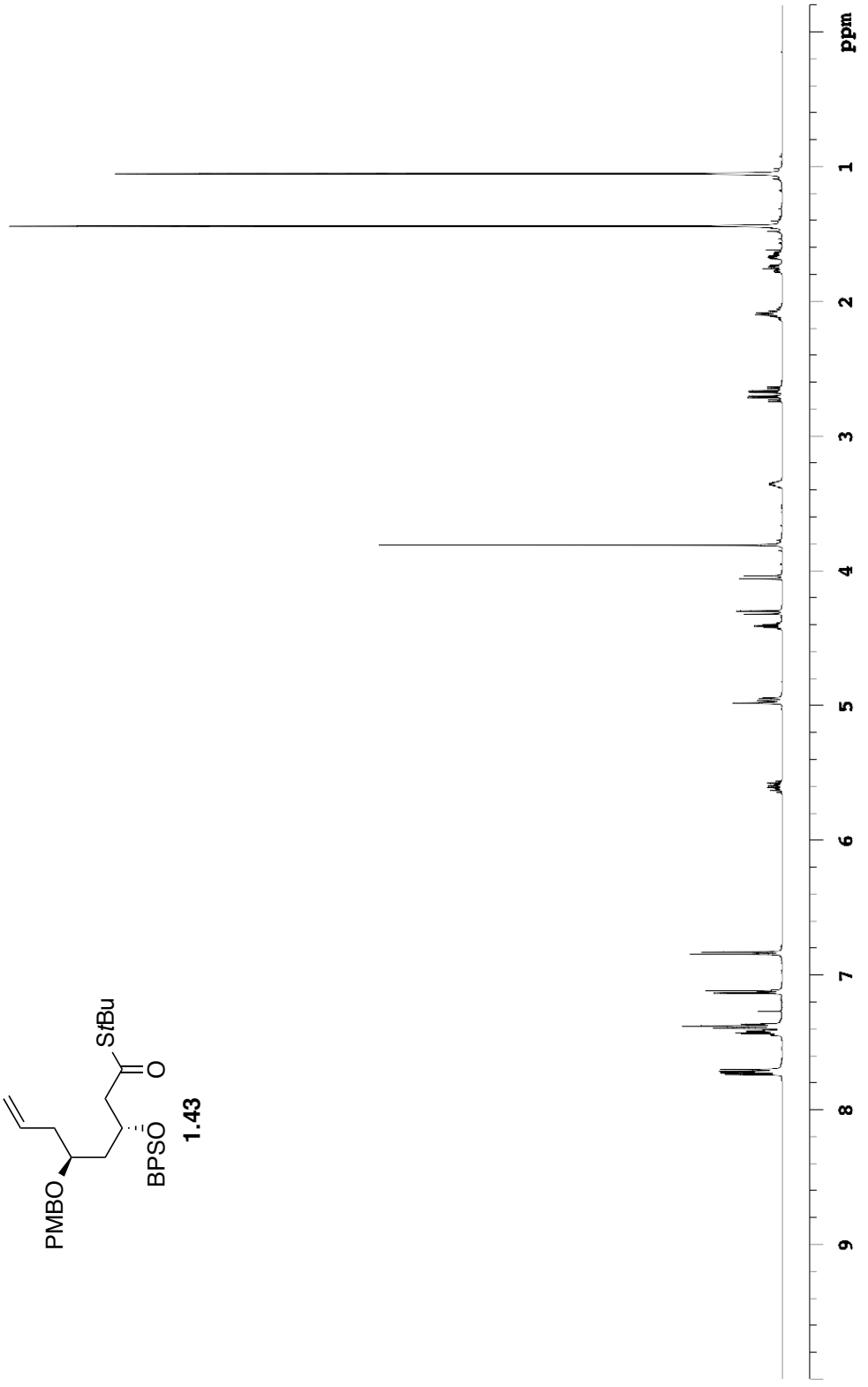


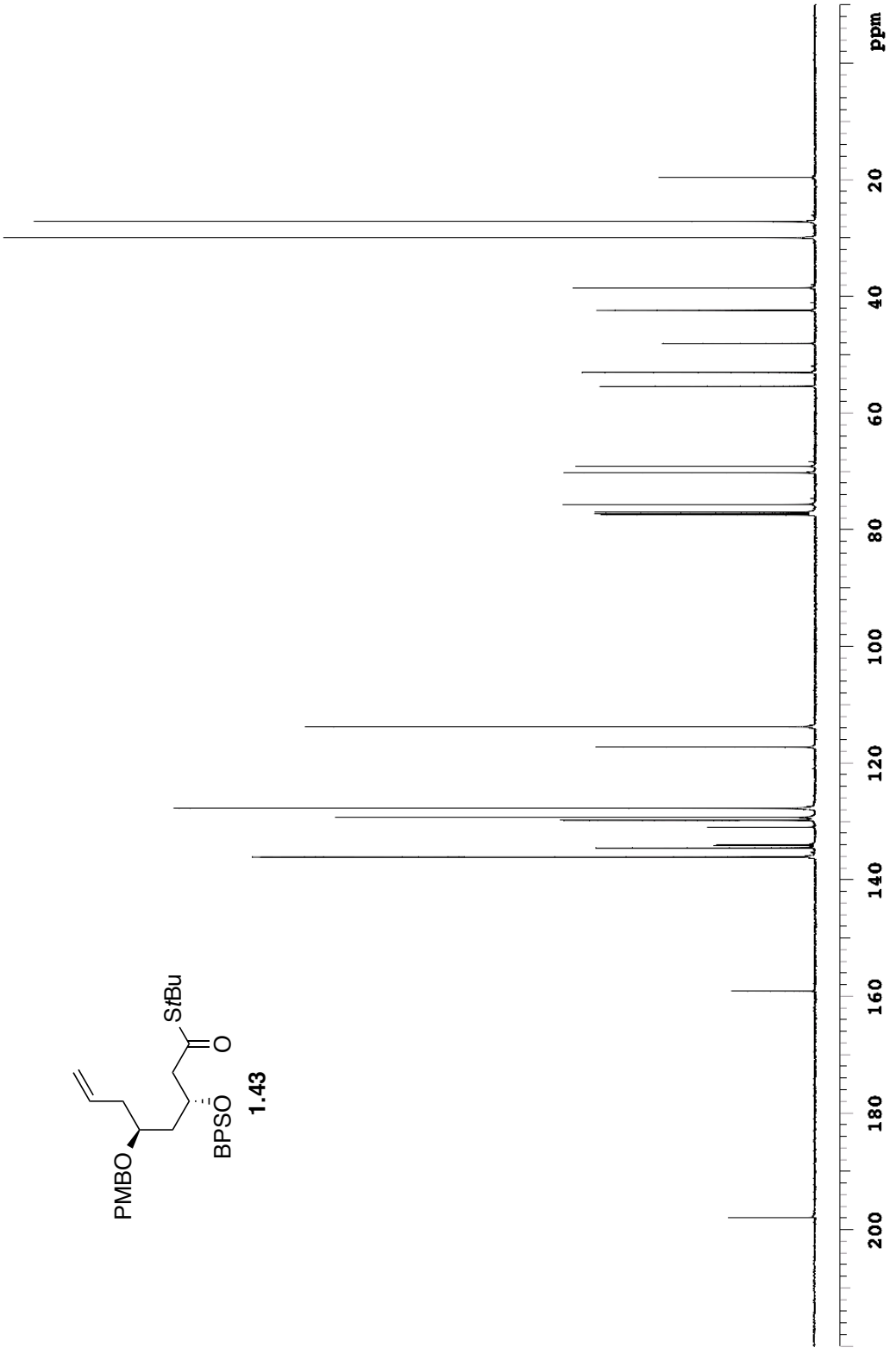
1.41

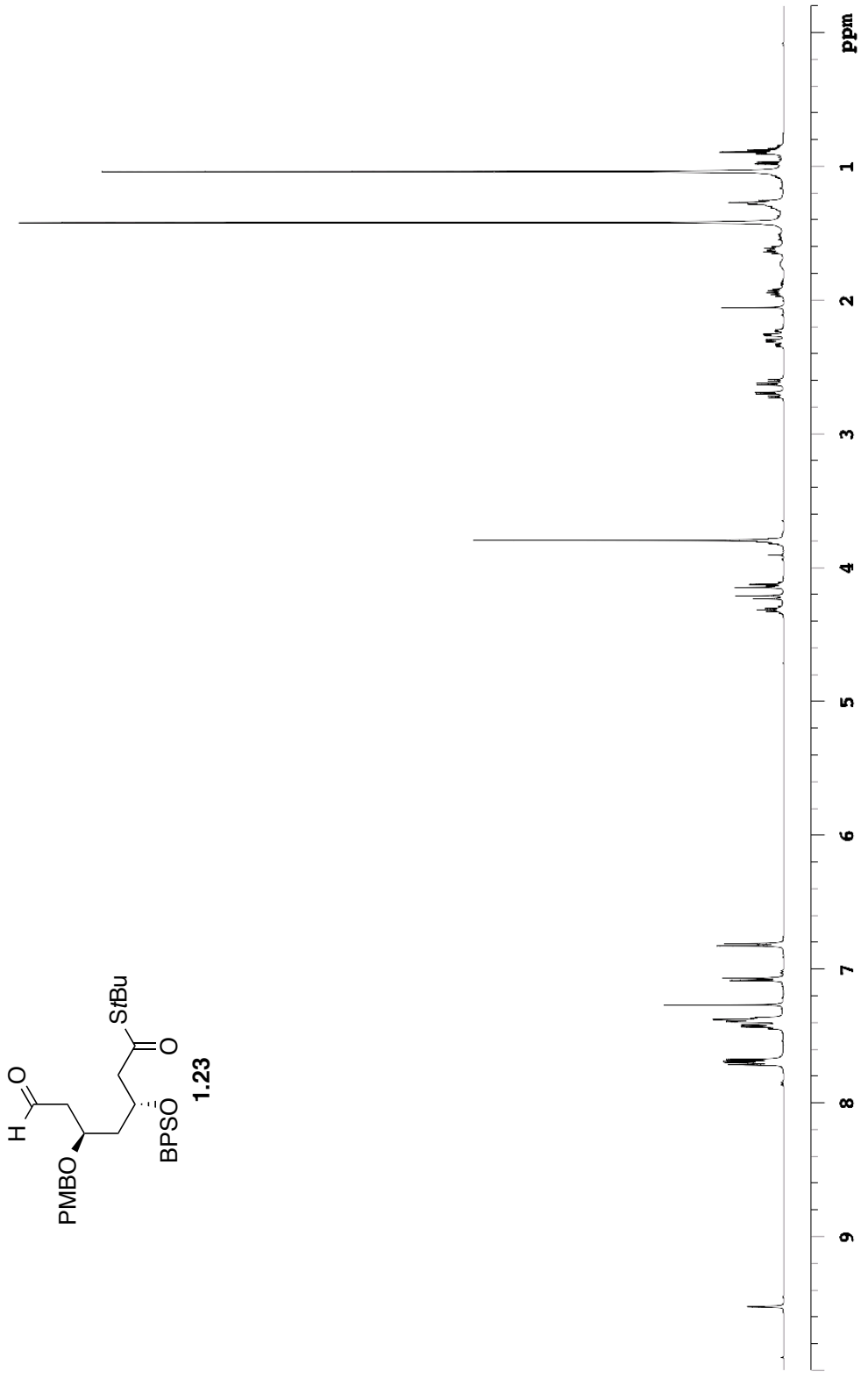

StBu
O
OH
PMBO
1.41
200
180
160
140
120
100
80
60
40
20
ppm

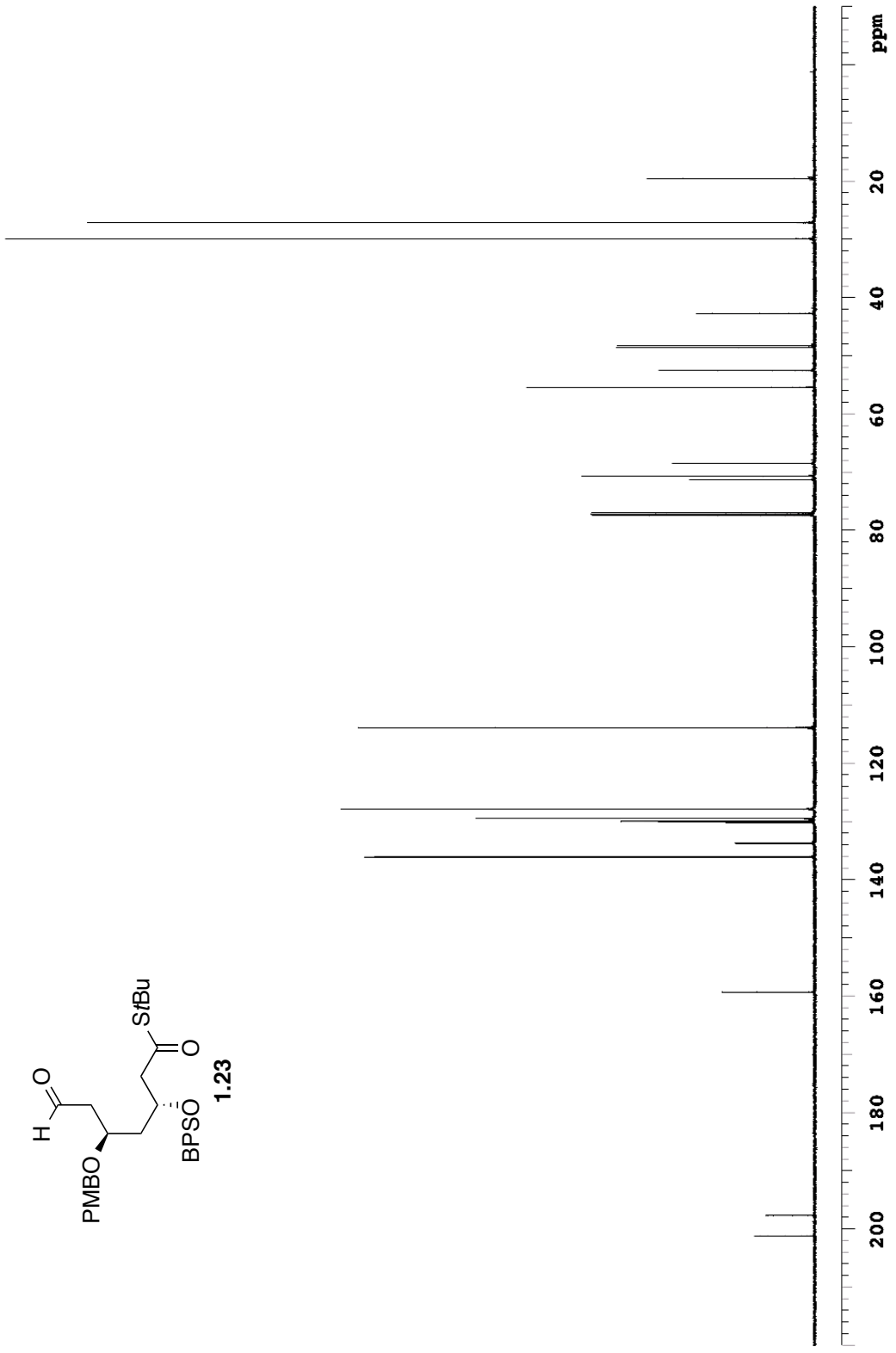

OMe
O
O
O
Me
Me
1.42
ppm
1
2
3
4
5
6
7
8
9

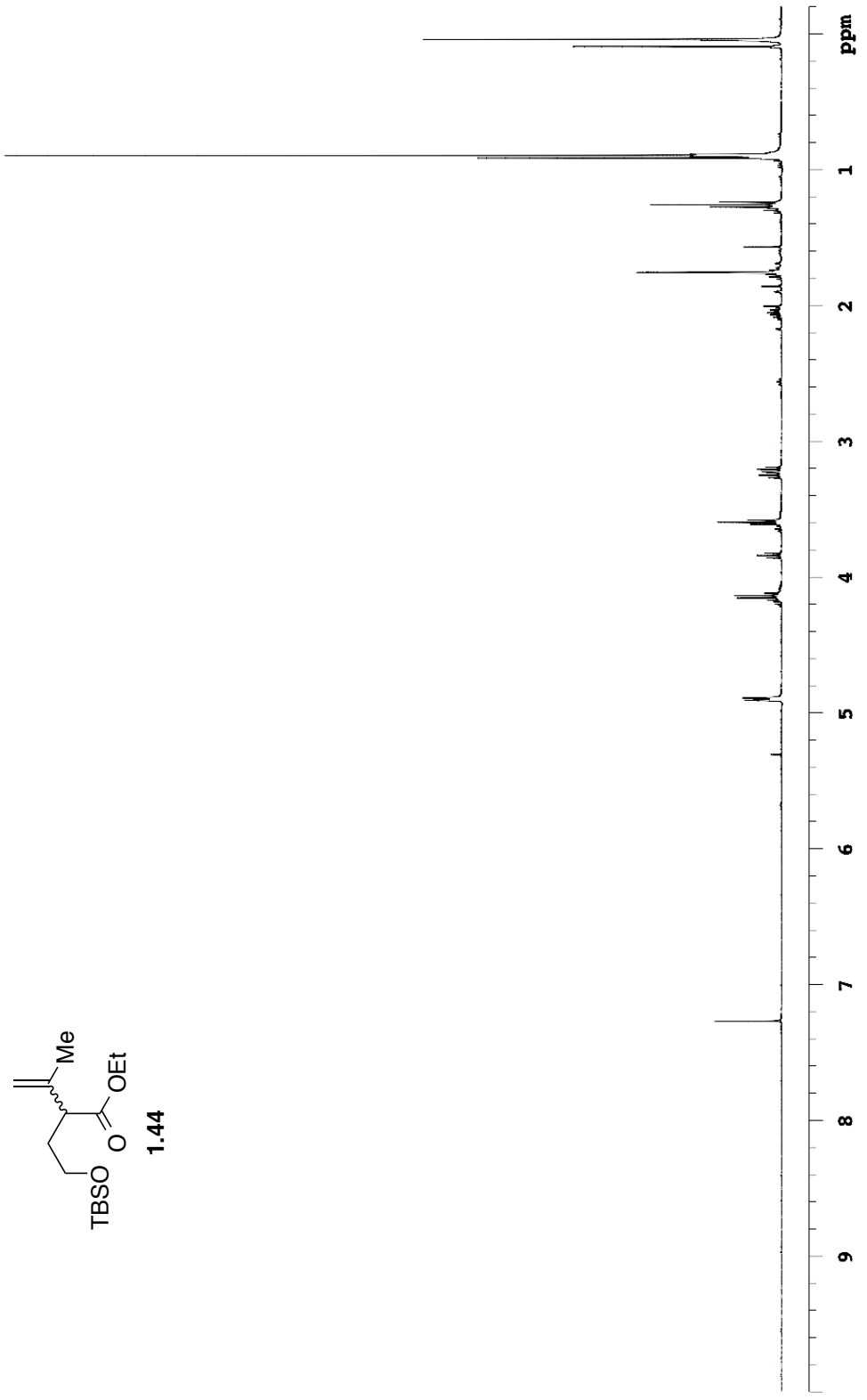

OMe
O
O
O
Me
Me
1.42
ppm
200
180
160
140
120
100
80
60
40
20

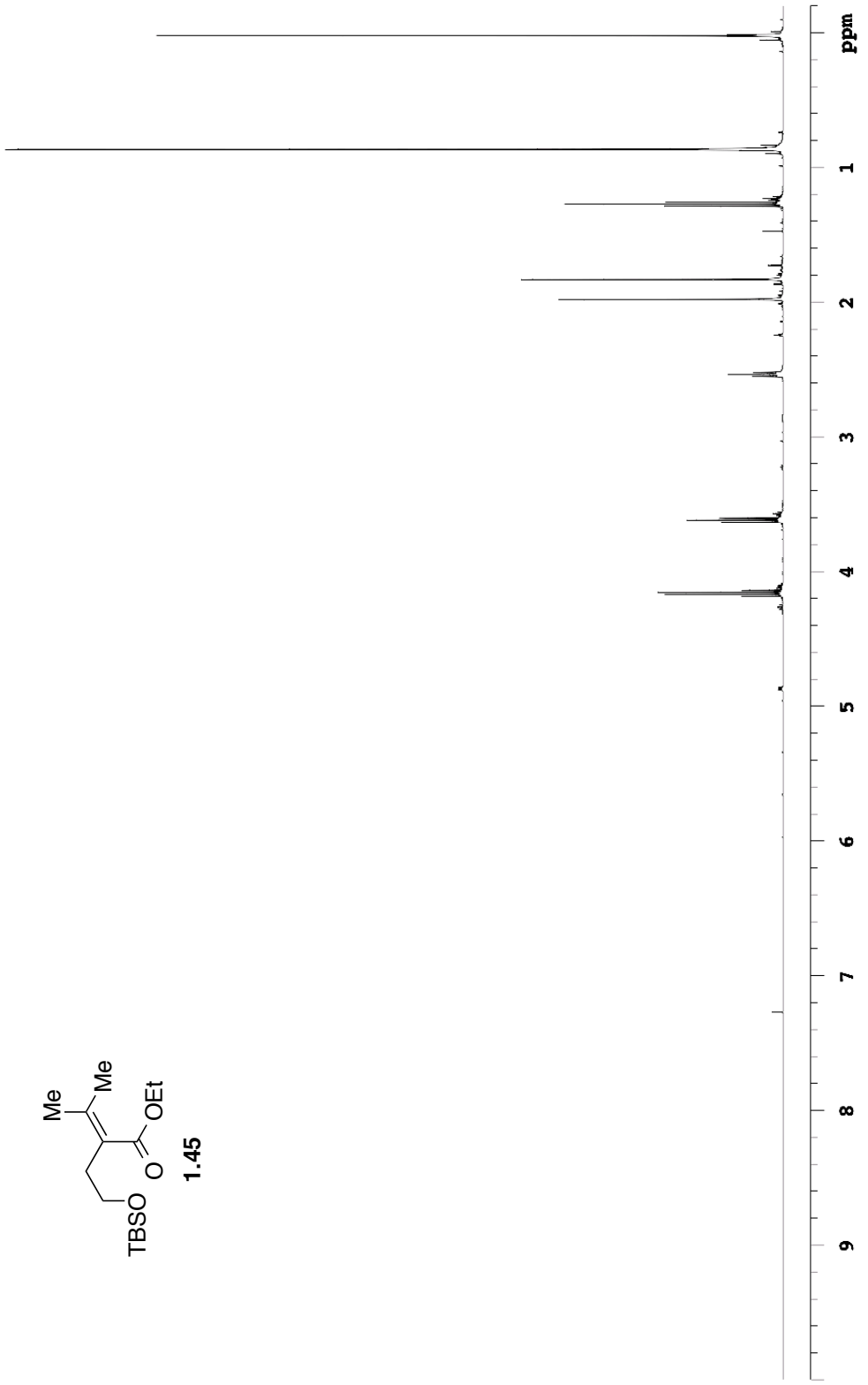

PMBO
BPSO
O
StBu
1.43
ppm
1
2
3
4
5
6
7
8
9


PMBO
BPSO
O
StBu
1.43
ppm
20
40
60
80
100
120
140
160
180
200

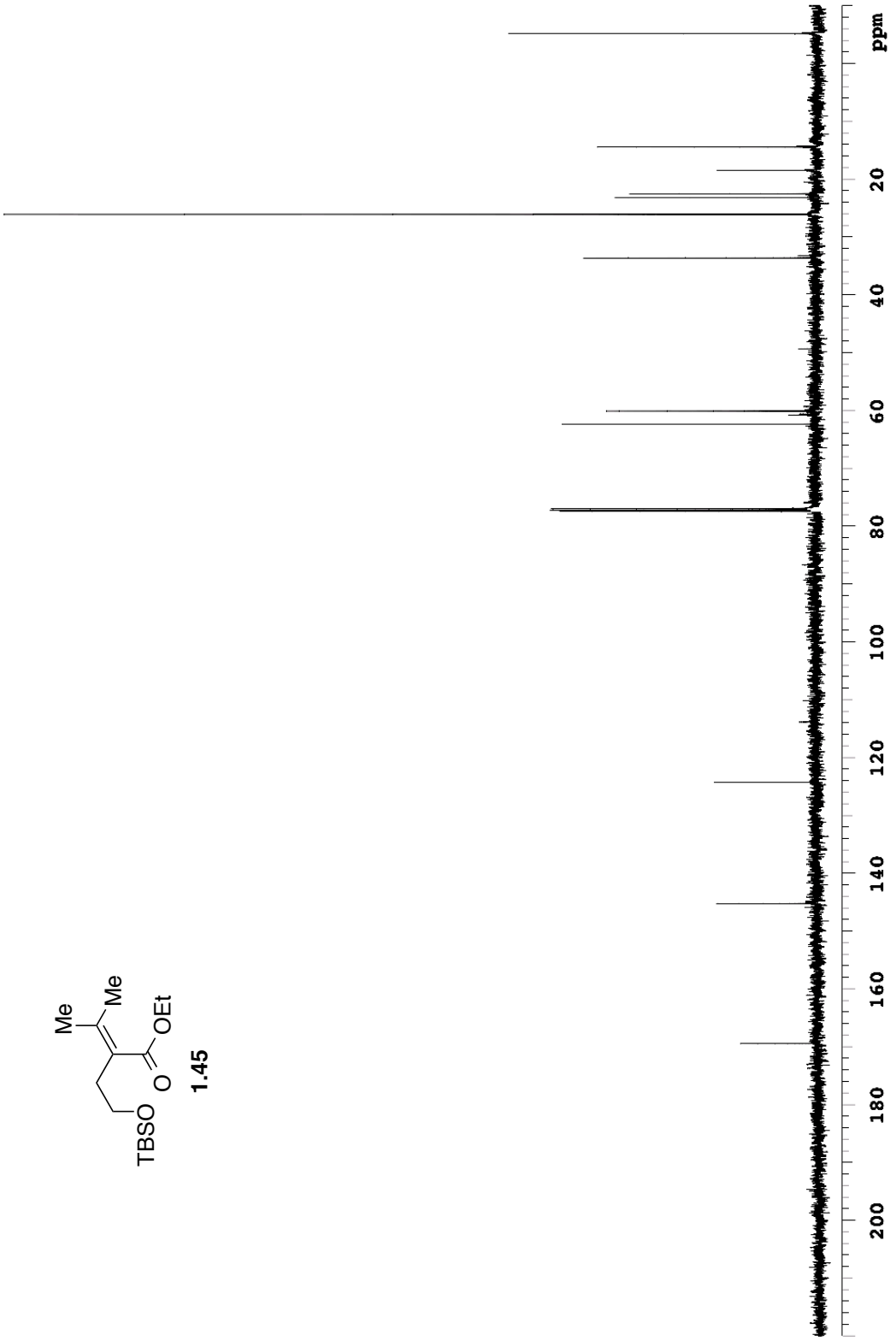

ppm
1
2
3
4
5
6
7
8
9
O
H
PMBO
BPSO
O
StBu
1.23


ppm
20
40
60
80
100
120
140
160
180
200
H
O
PMBO
BPSO
O
O
StBu
1.23

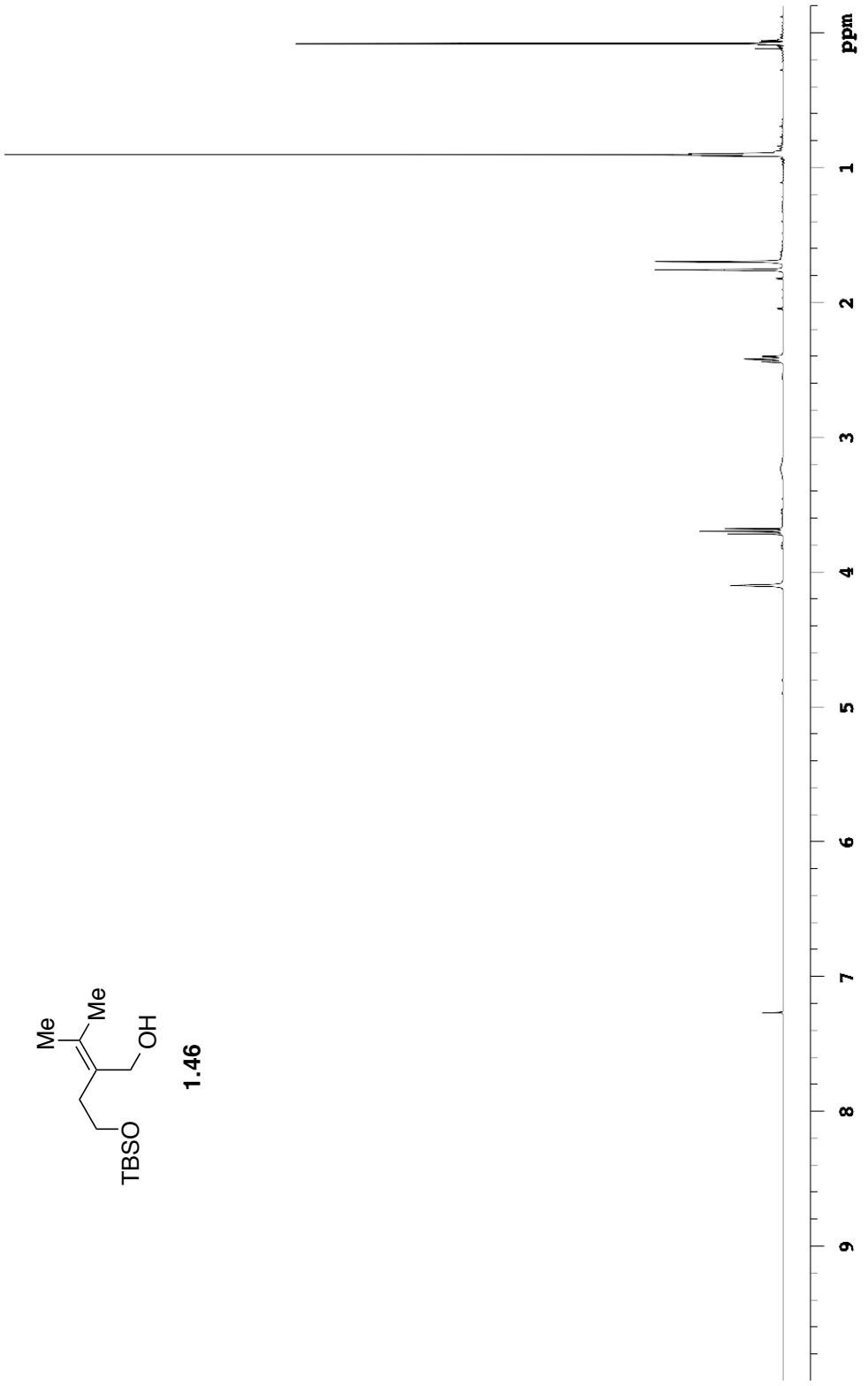

Me
OEt
O
TBSO
1.44
ppm
1
2
3
4
5
6
7
8
9

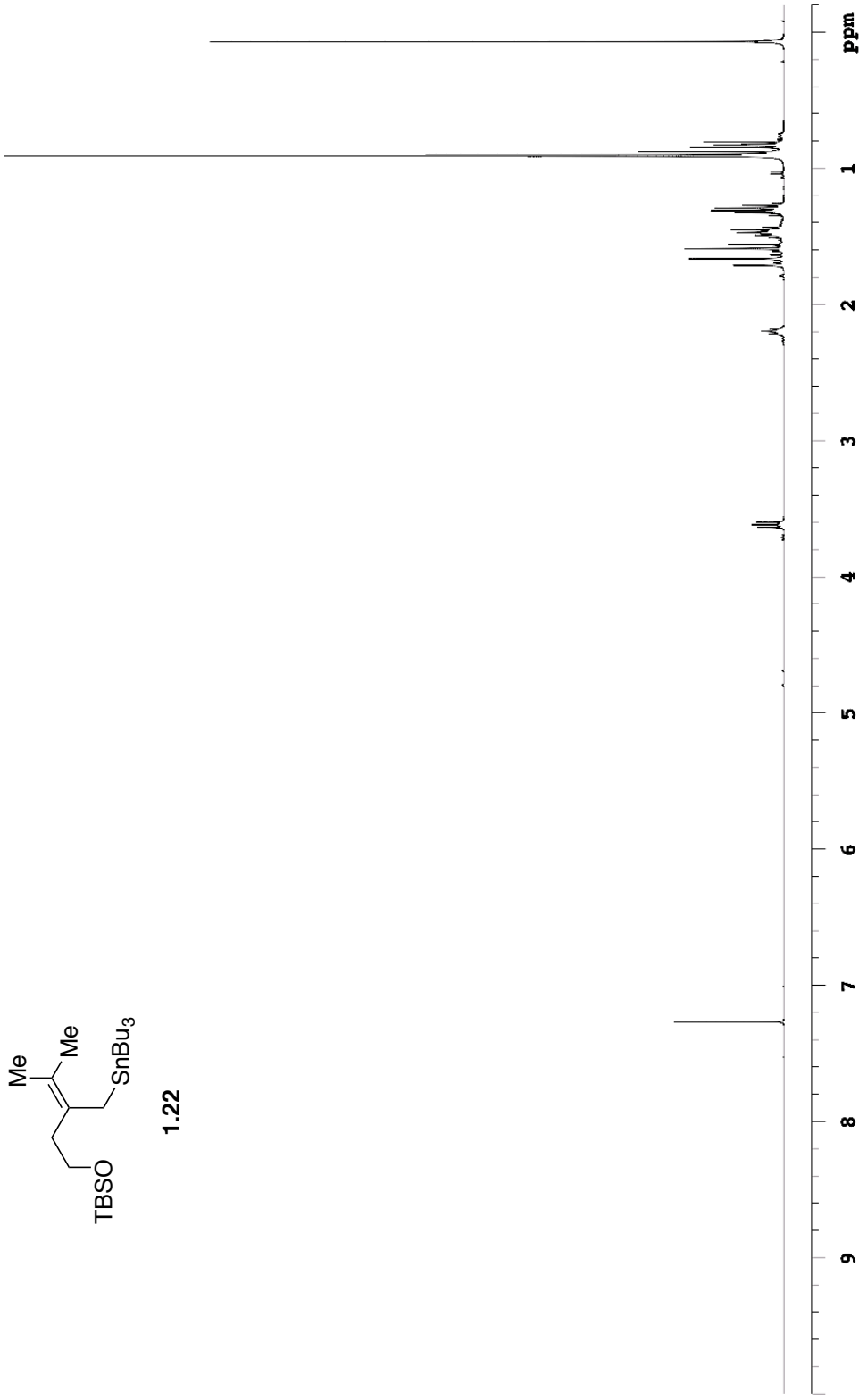


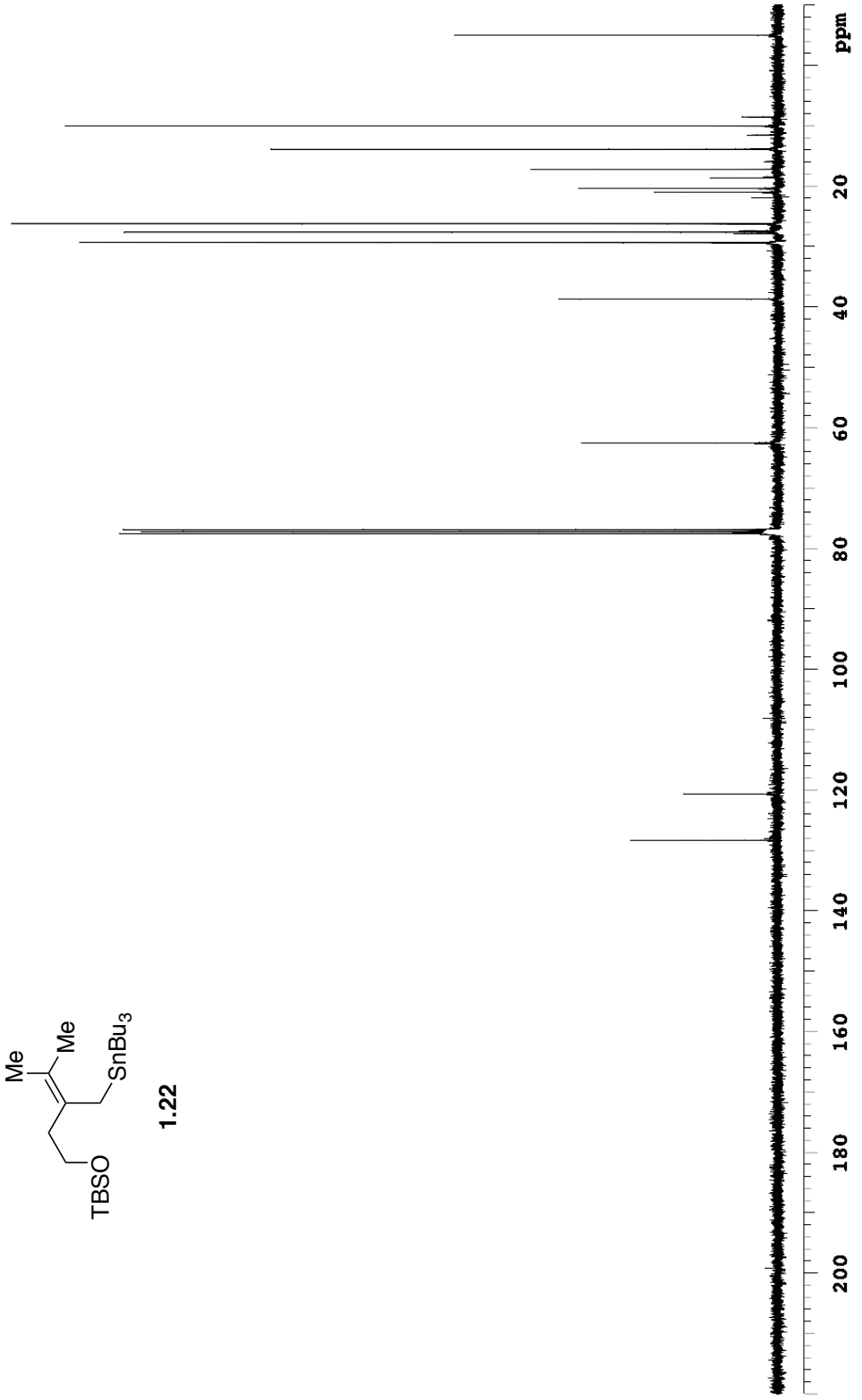
1.45

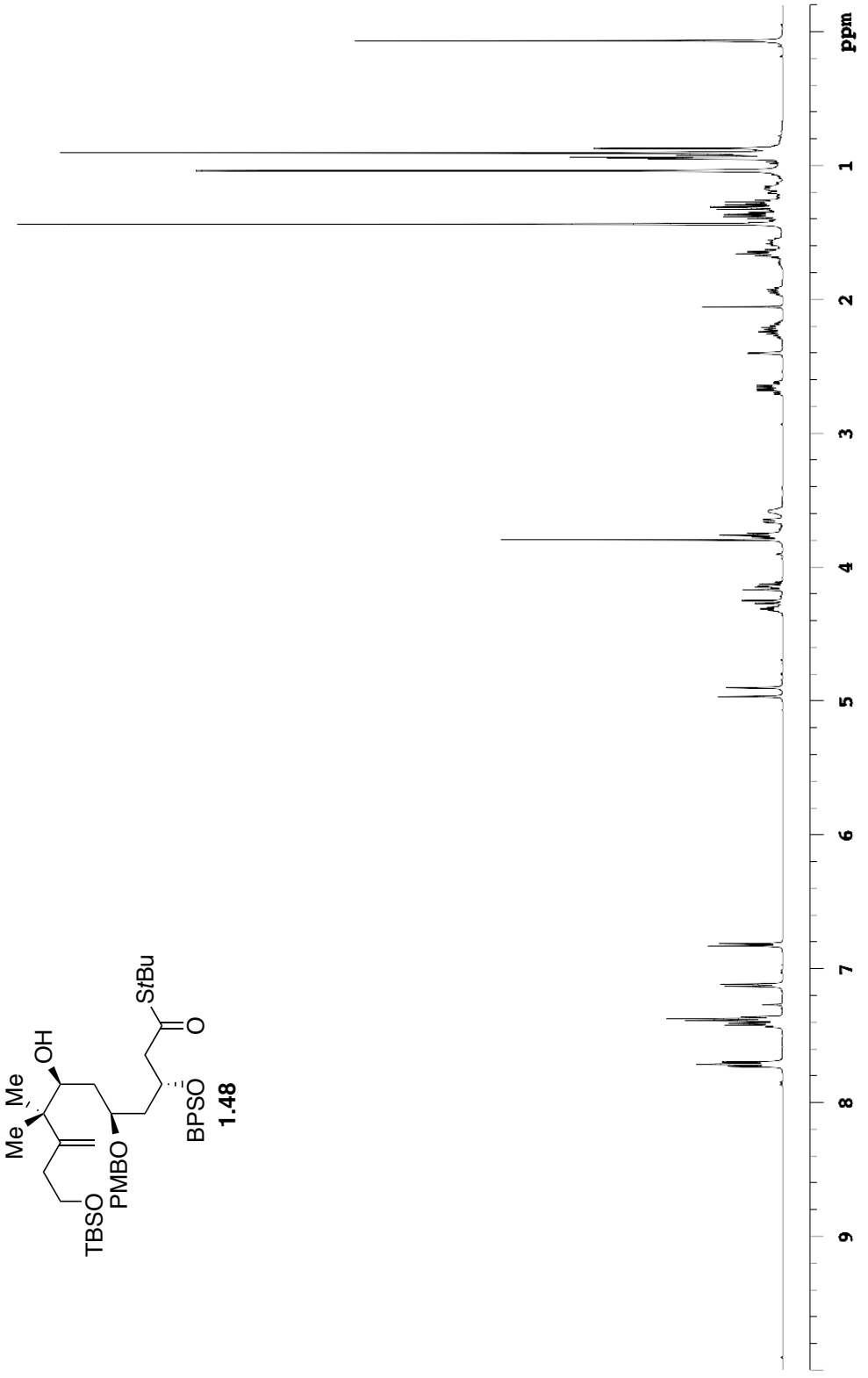


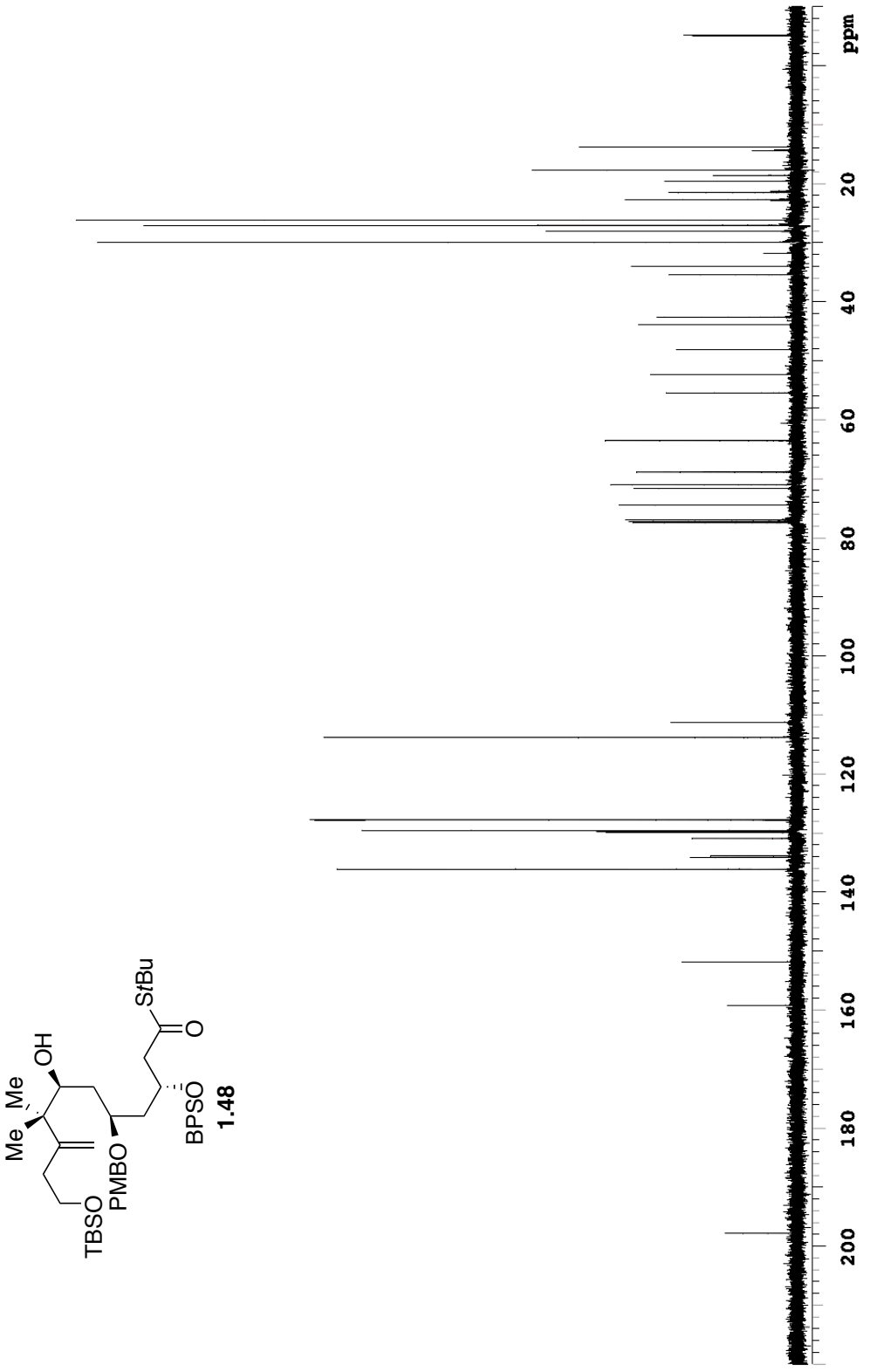
1.45

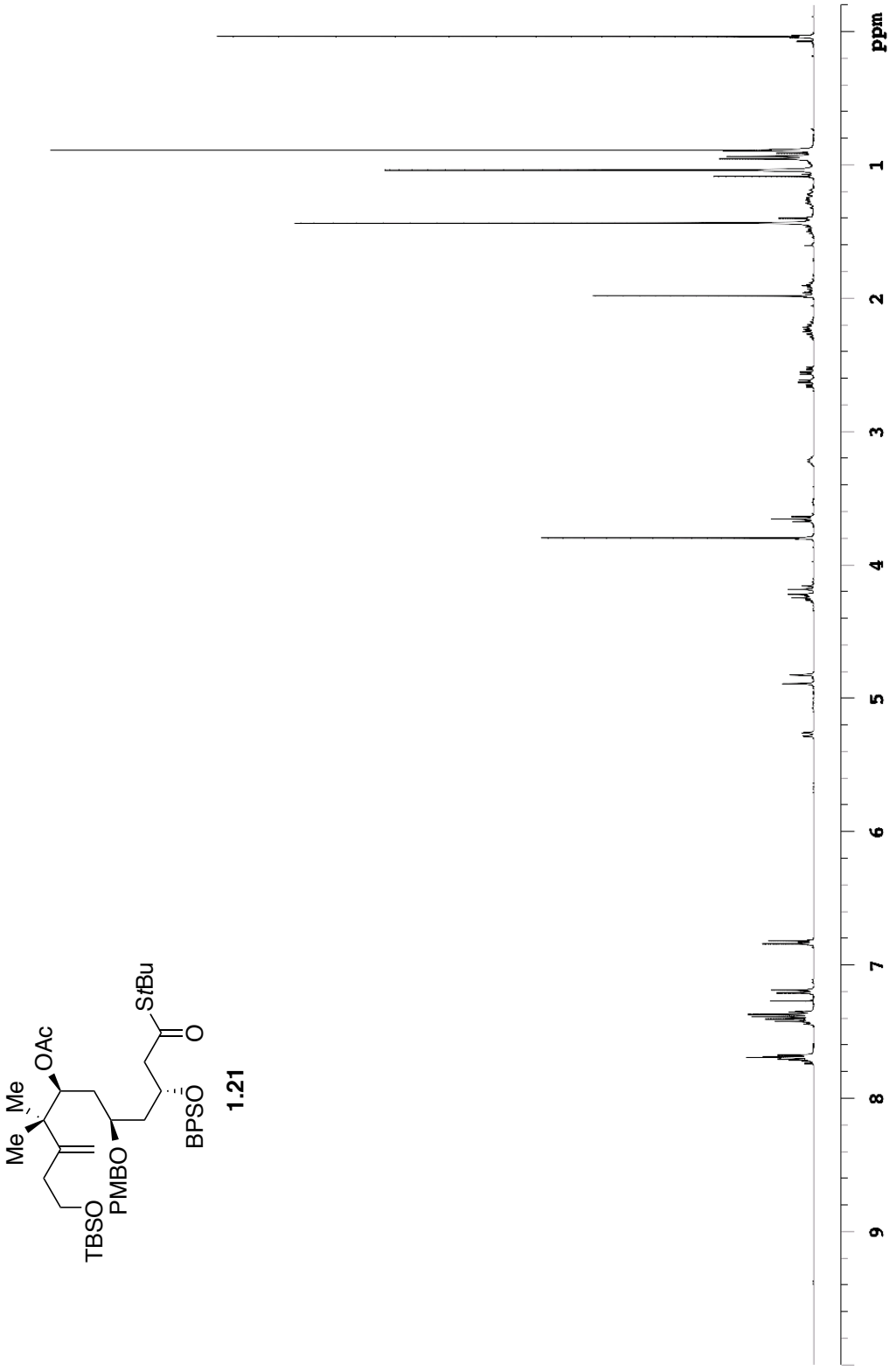

Me
Me
OH
TBSO
1.46
ppm
1
2
3
4
5
6
7
8
9

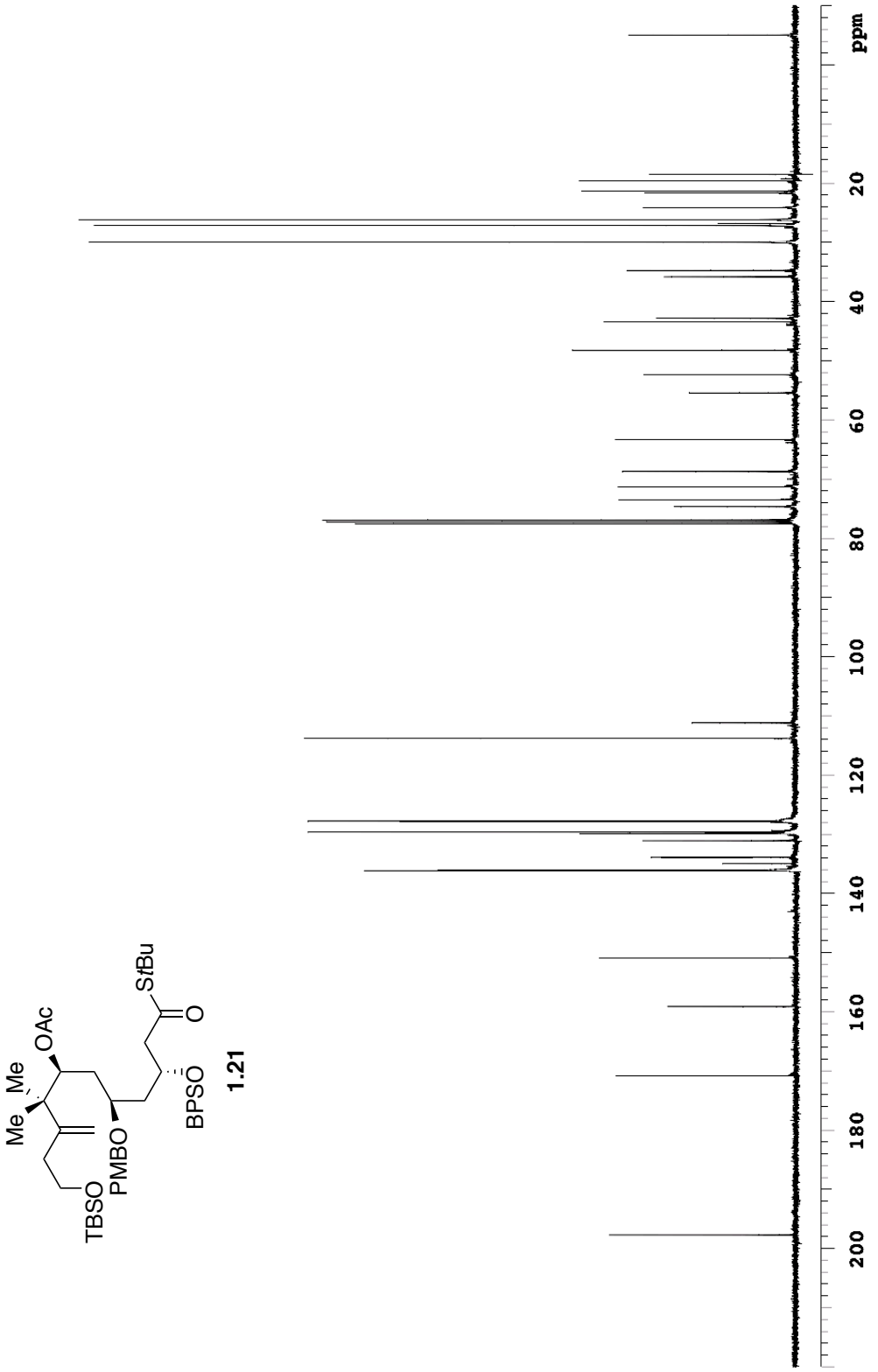

Me
Me
SnBu3
TBSO
1.22
ppm
1
2
3
4
5
6
7
8
9

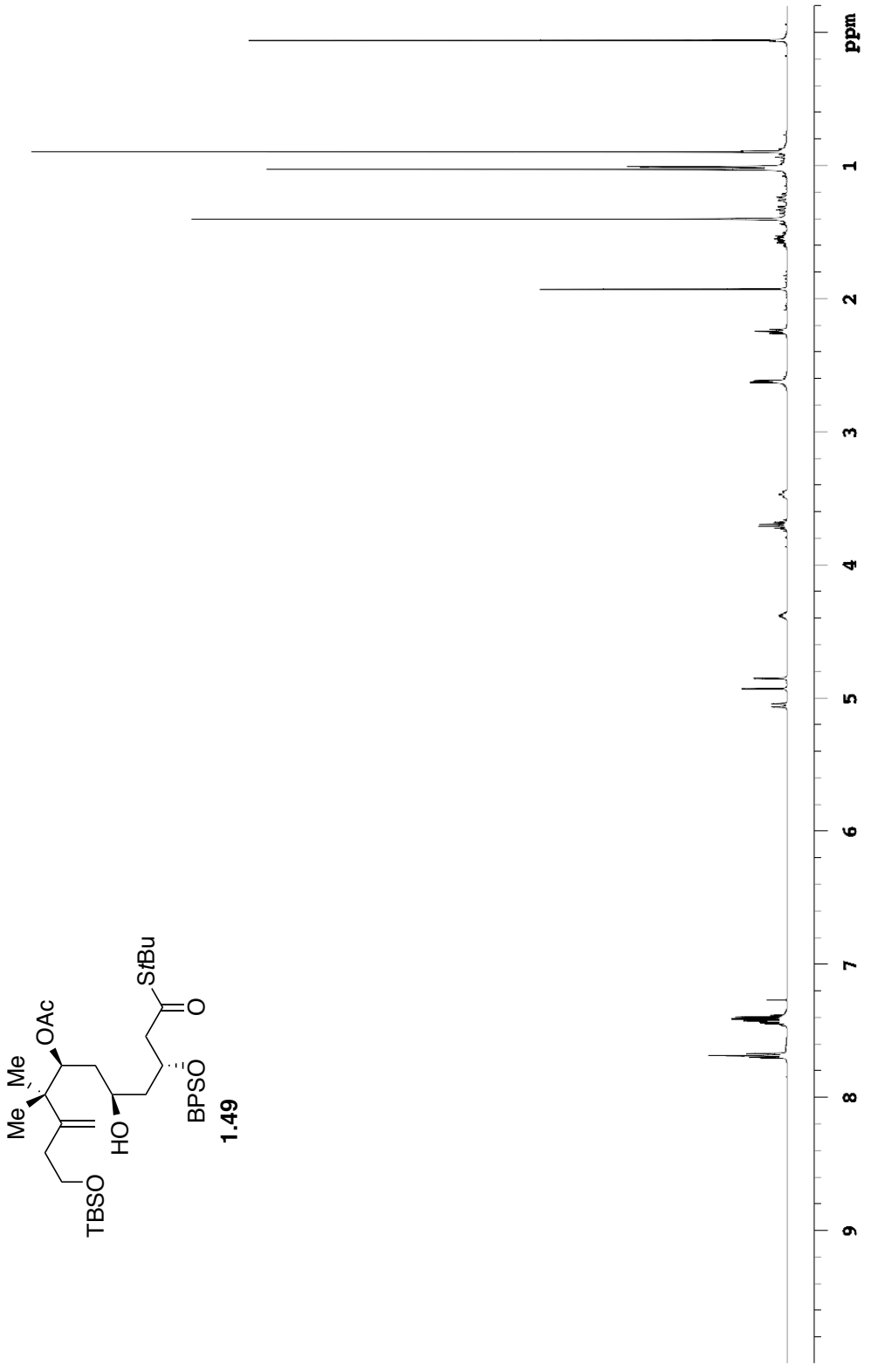

Me
Me
SnBu3
TBSO
1.22
ppm
200
180
160
140
120
100
80
60
40
20

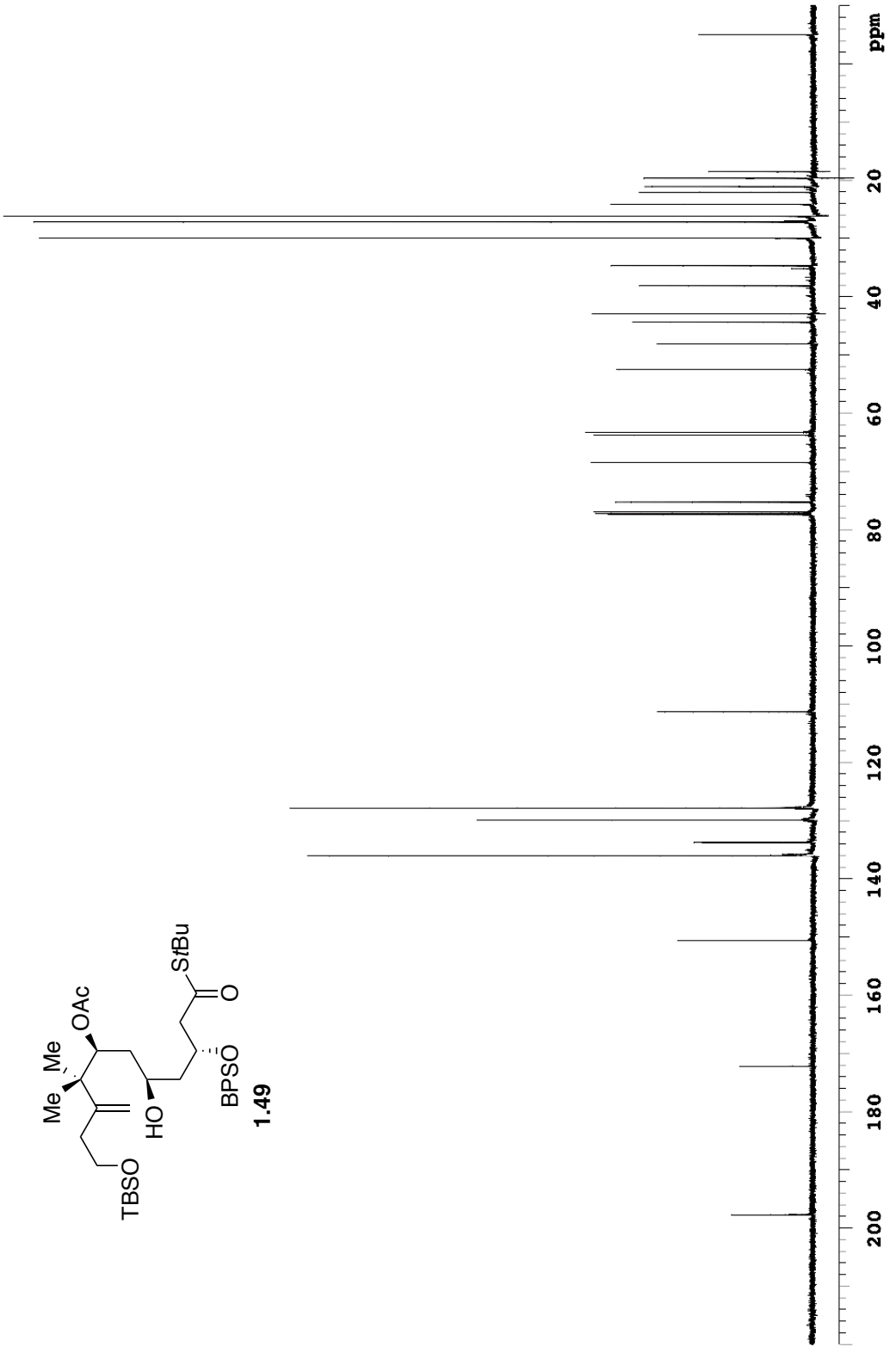

StBu
O
OH
Me
Me
BPSO
PMBO
TBSO
1.48
ppm
1
2
3
4
5
6
7
8
9


Me
Me
OH
TBSO
PMBO
BPSO
O
StBu
1.48
ppm
20
40
60
80
100
120
140
160
180
200

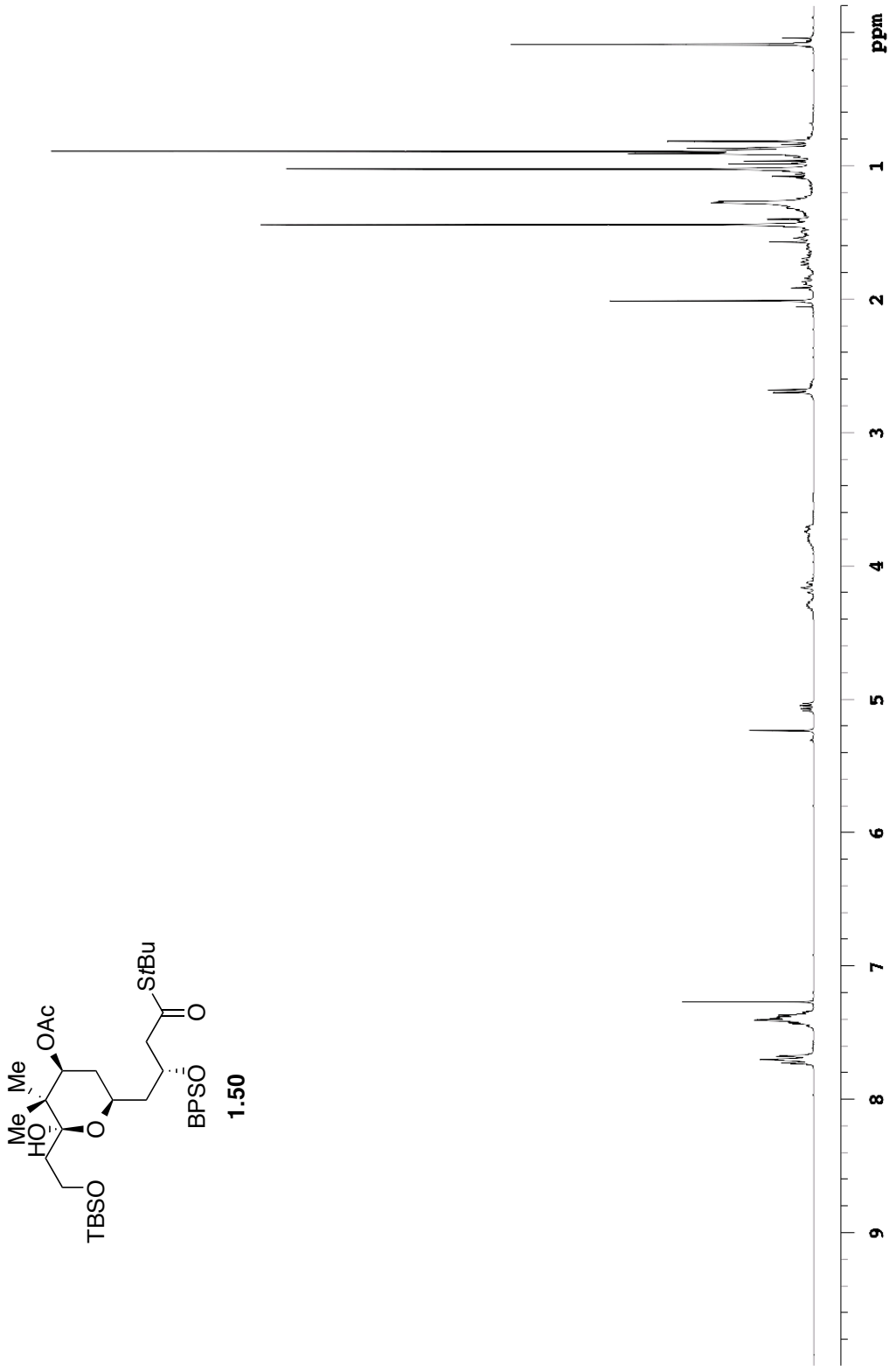

Me
Me
OAc
TBSO
PMBO
BPSO
O
StBu
1.21
ppm
1
2
3
4
5
6
7
8
9

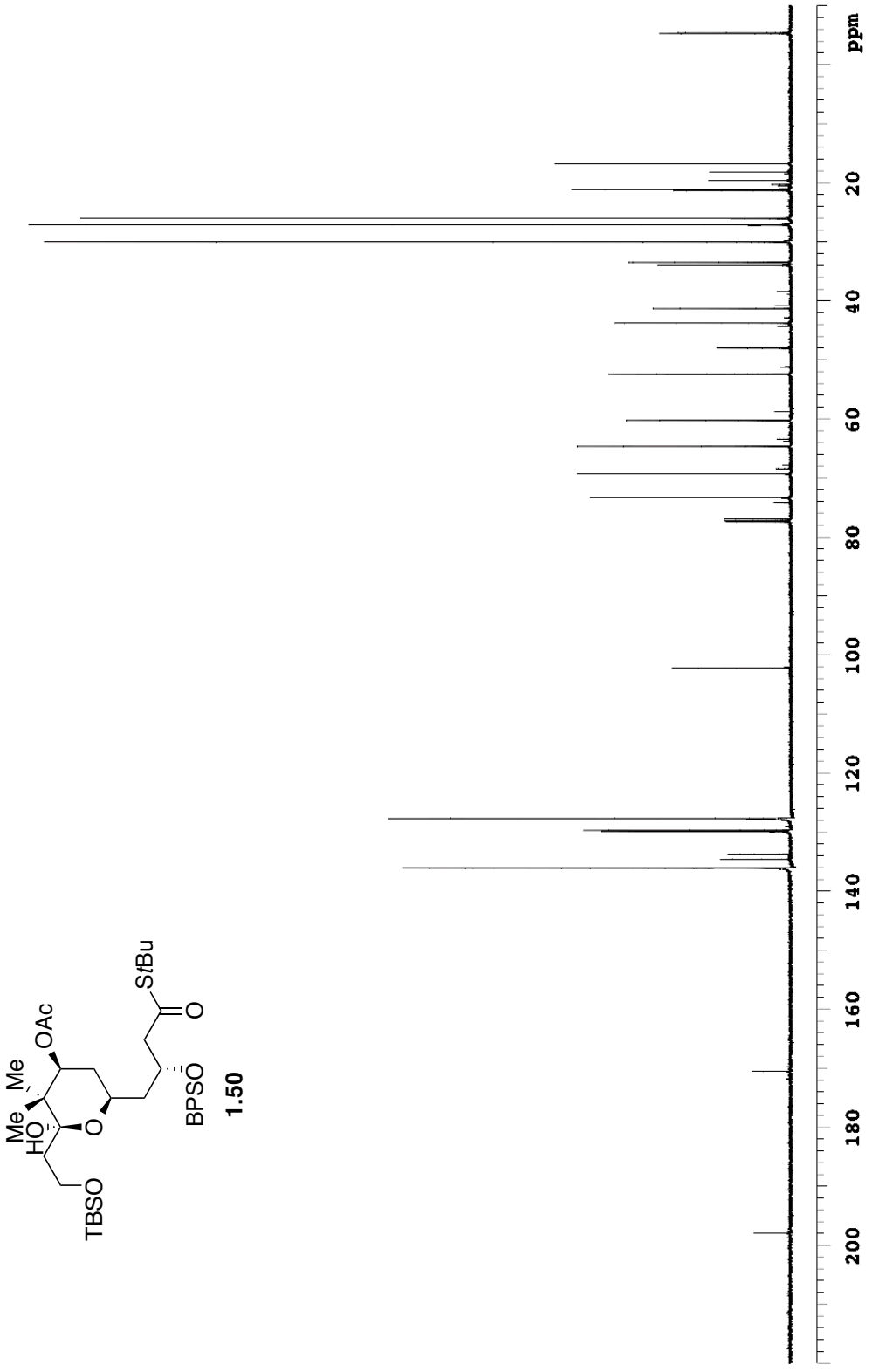

Me
Me
OAc
TBSO
PMBO
BPSO
O
StBu
1.21
ppm
20
40
60
80
100
120
140
160
180
200


StBu
O
OAc
Me
Me
BPSO
HO
TBSO
1.49
ppm
9
8
7
6
5
4
3
2
1

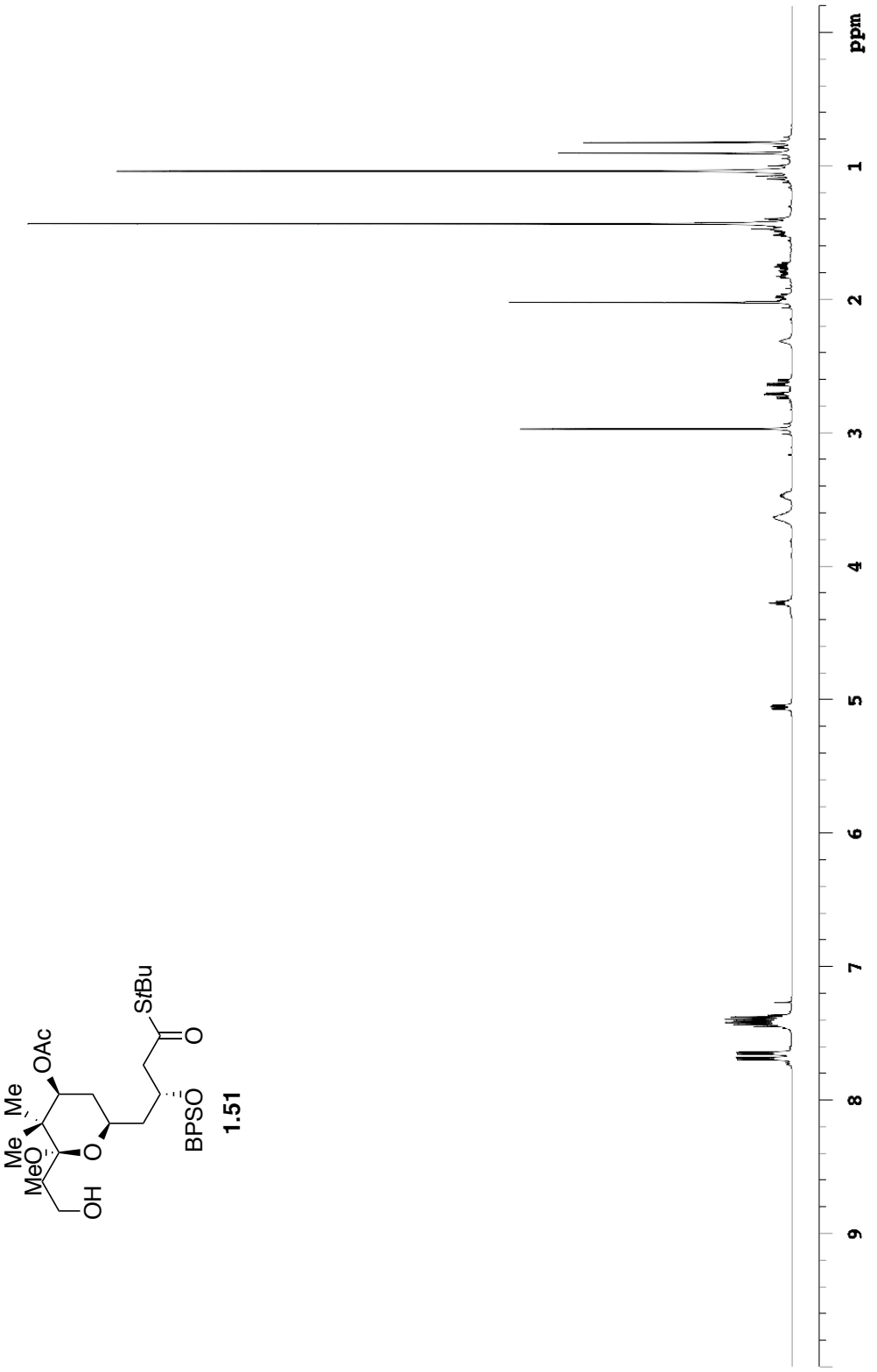

ppm
20
40
60
80
100
120
140
160
180
200
TBSO
HO
BPSO
OAc
Me
Me
StBu
O

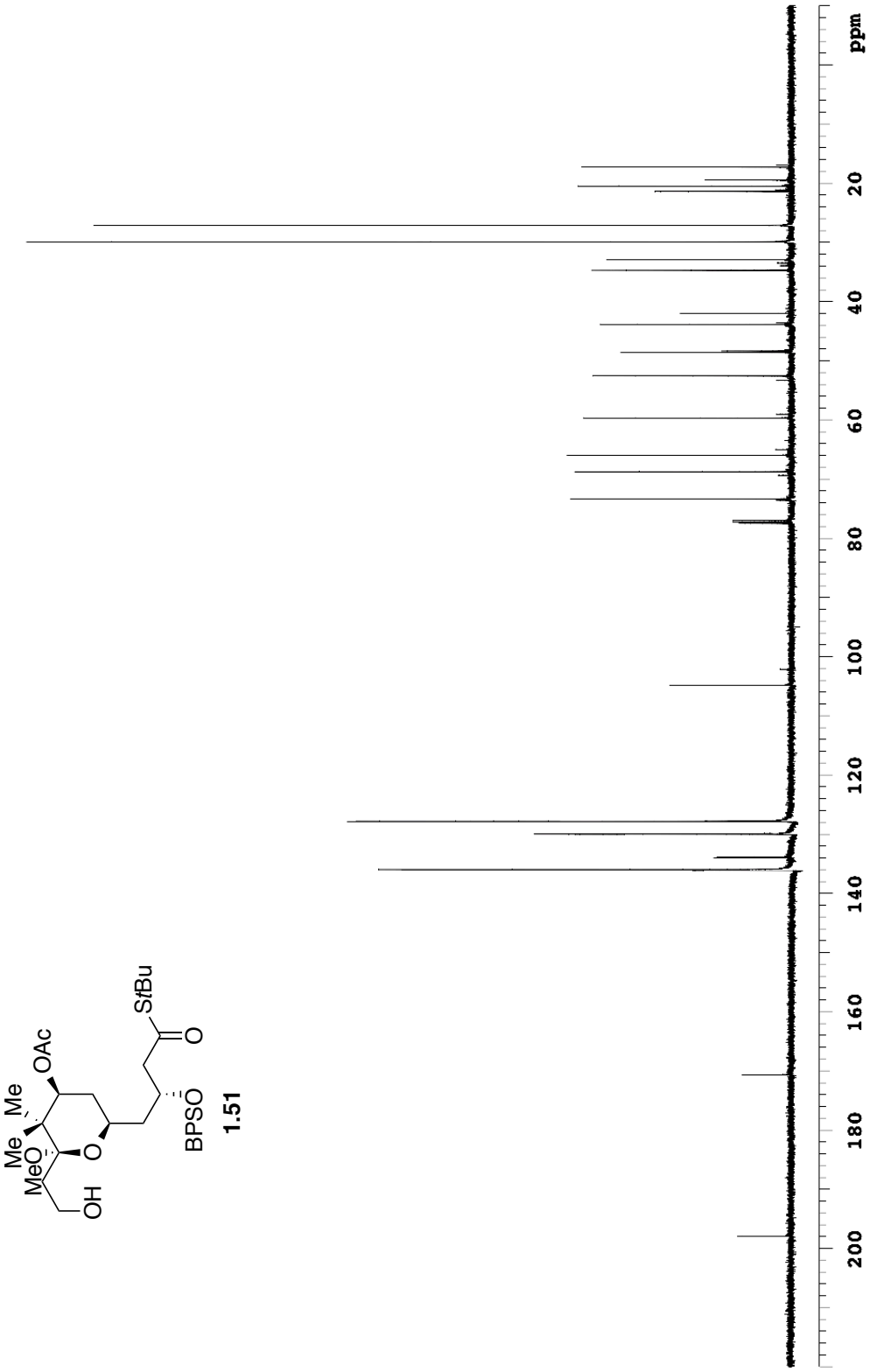
1.49

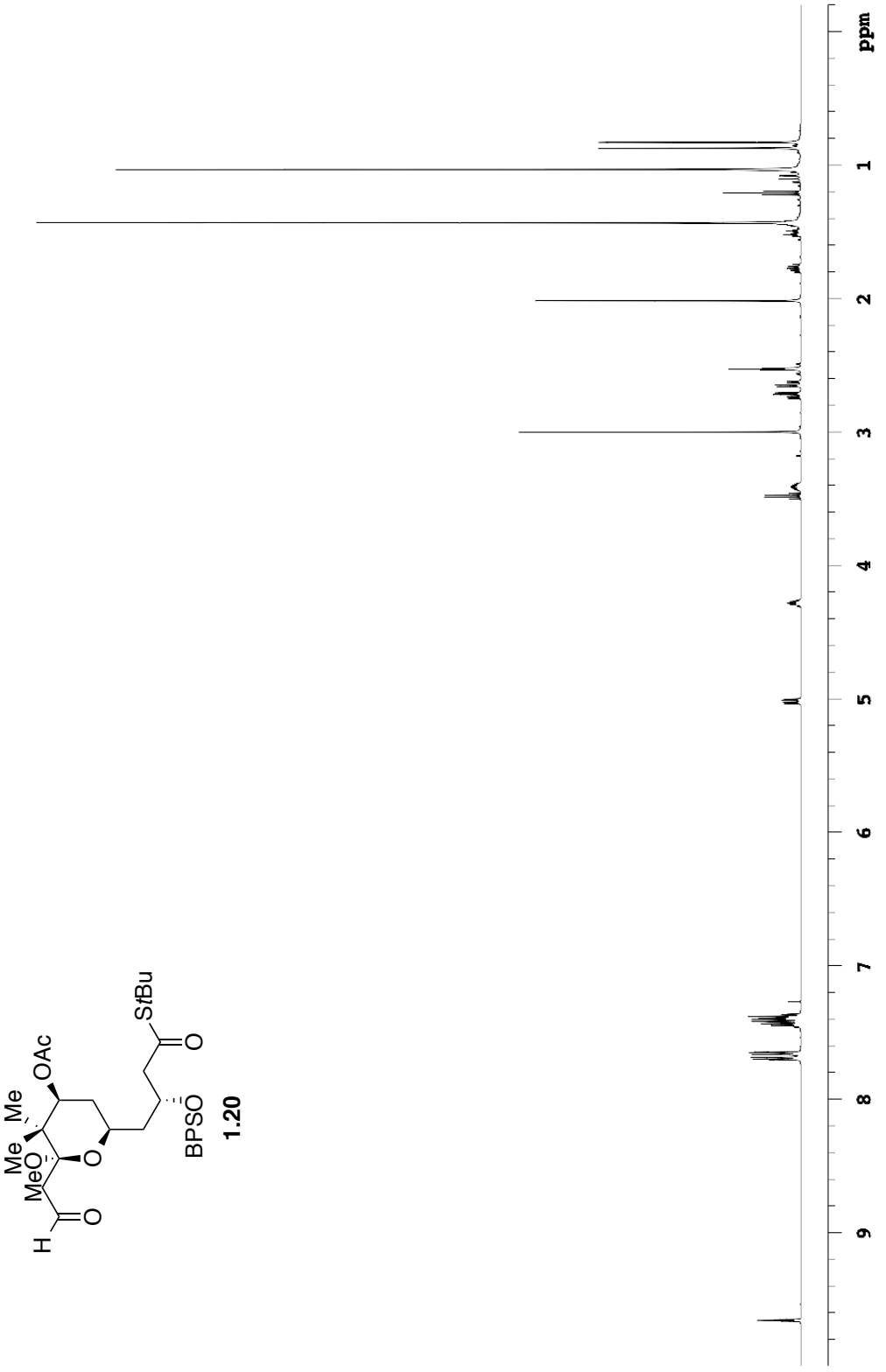

TBSO
HO
Me
Me
OAc
O
BPSO
O
StBu
1.50
ppm
1
2
3
4
5
6
7
8
9


ppm
20
40
60
80
100
120
140
160
180
200
StBu
O
OAc
Me
Me
HO
O
BPSO
TBSO

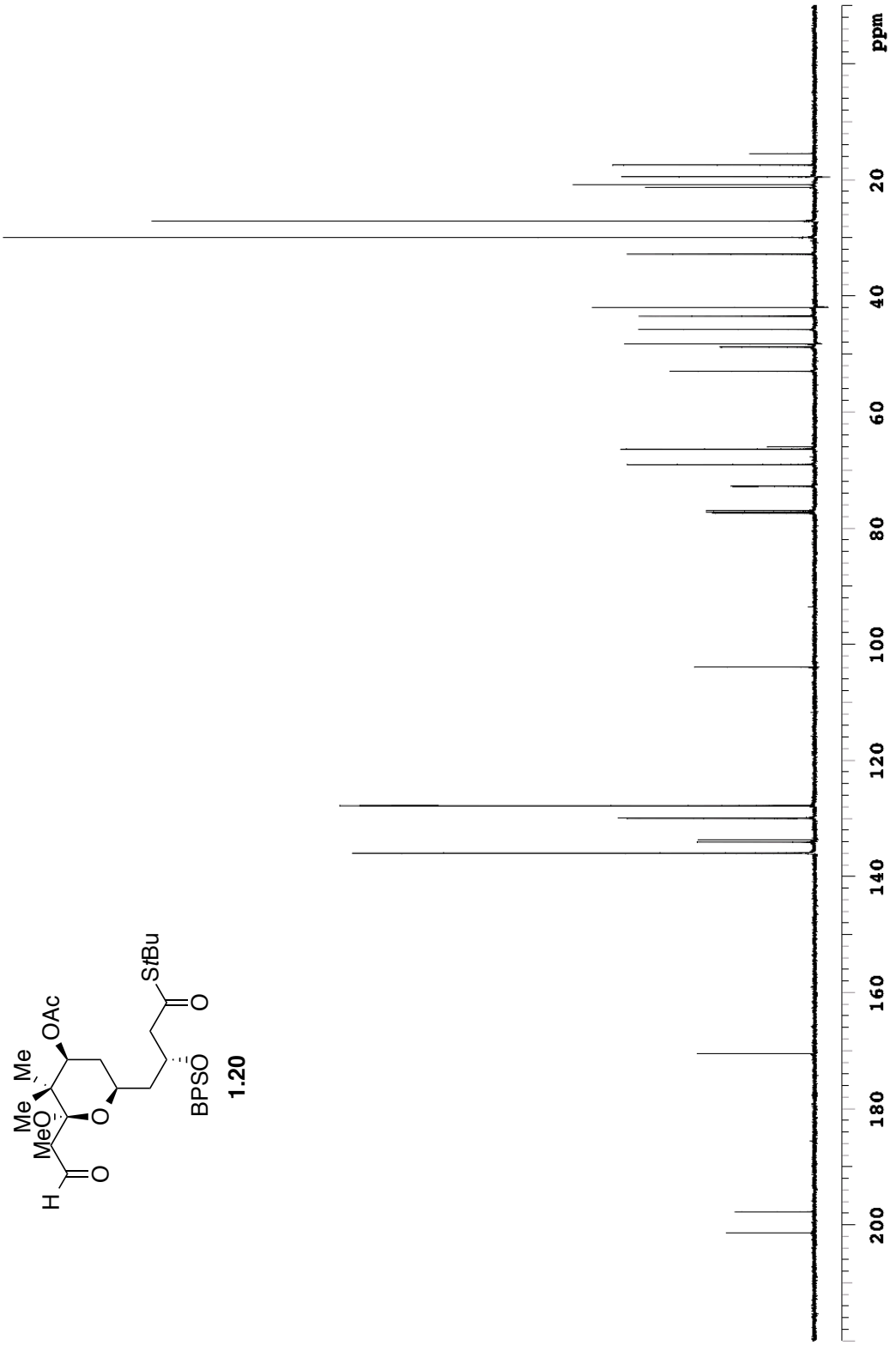
1.50

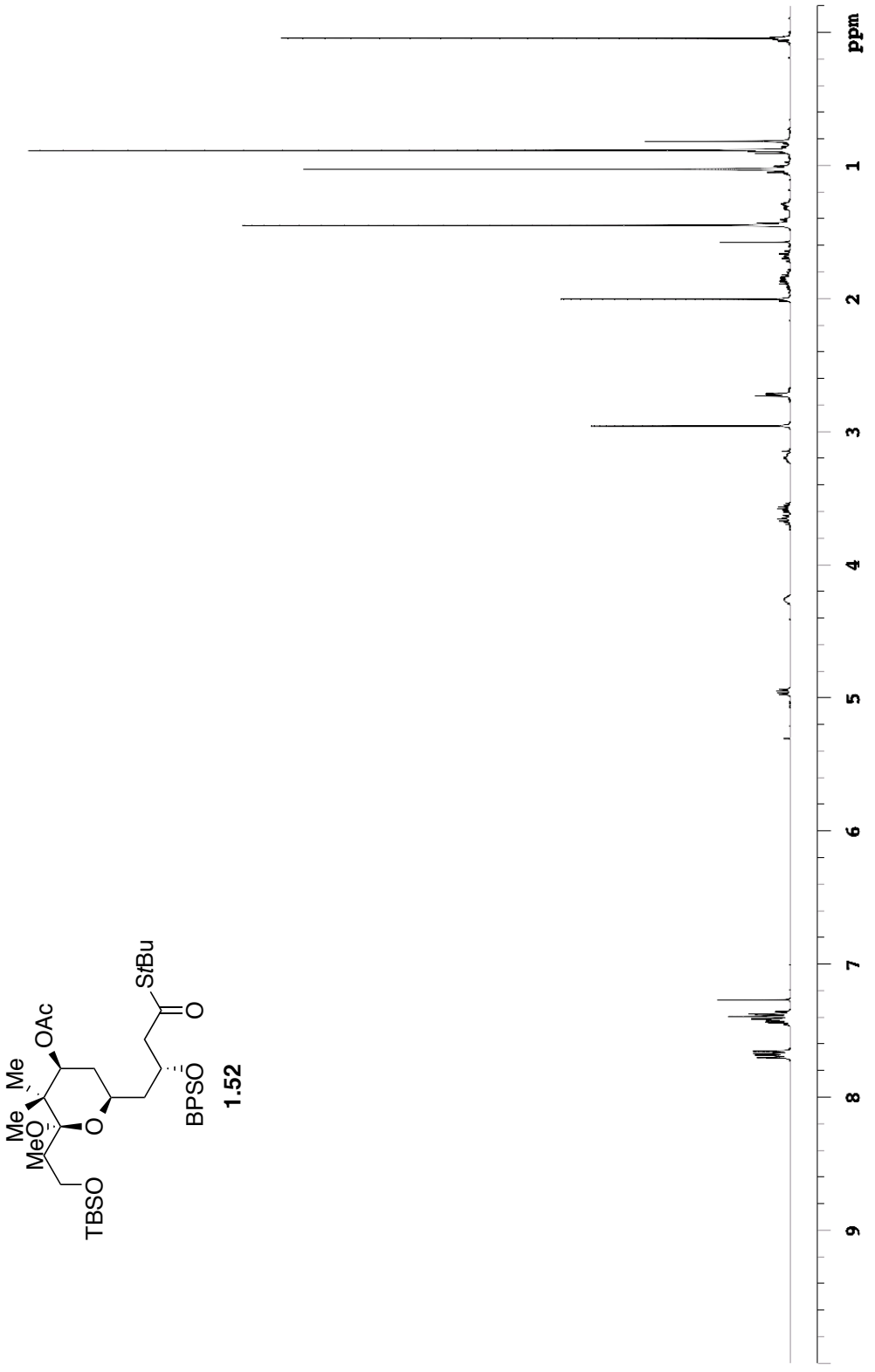

StBu
O
OAc
Me
Me
MeO
O
OH
BPSO
1.51
ppm
1
2
3
4
5
6
7
8
9

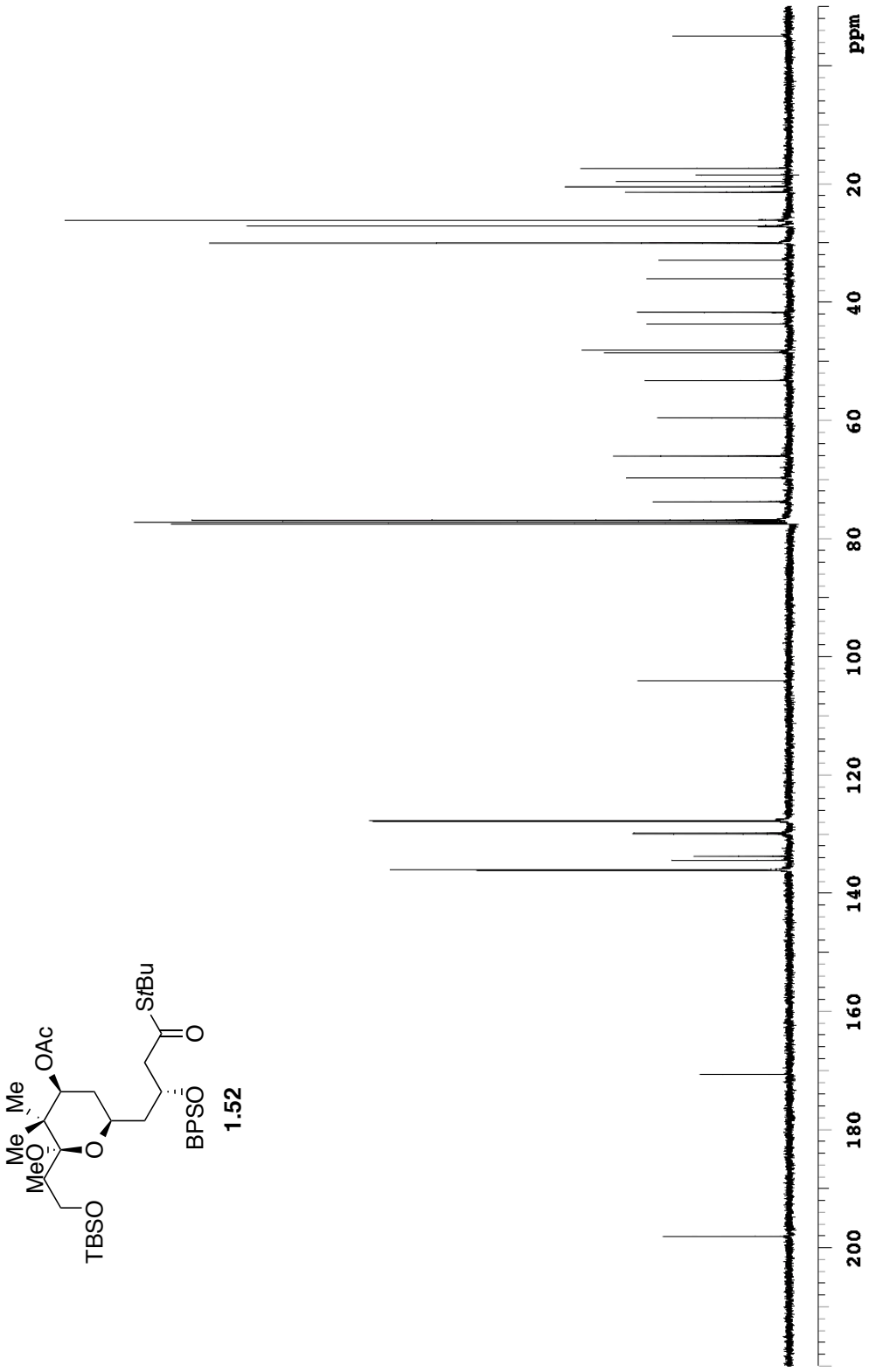

Me
Me
OAc
MeO
O
OH
BPSO
StBu
O
1.51
ppm
20
40
60
80
100
120
140
160
180
200

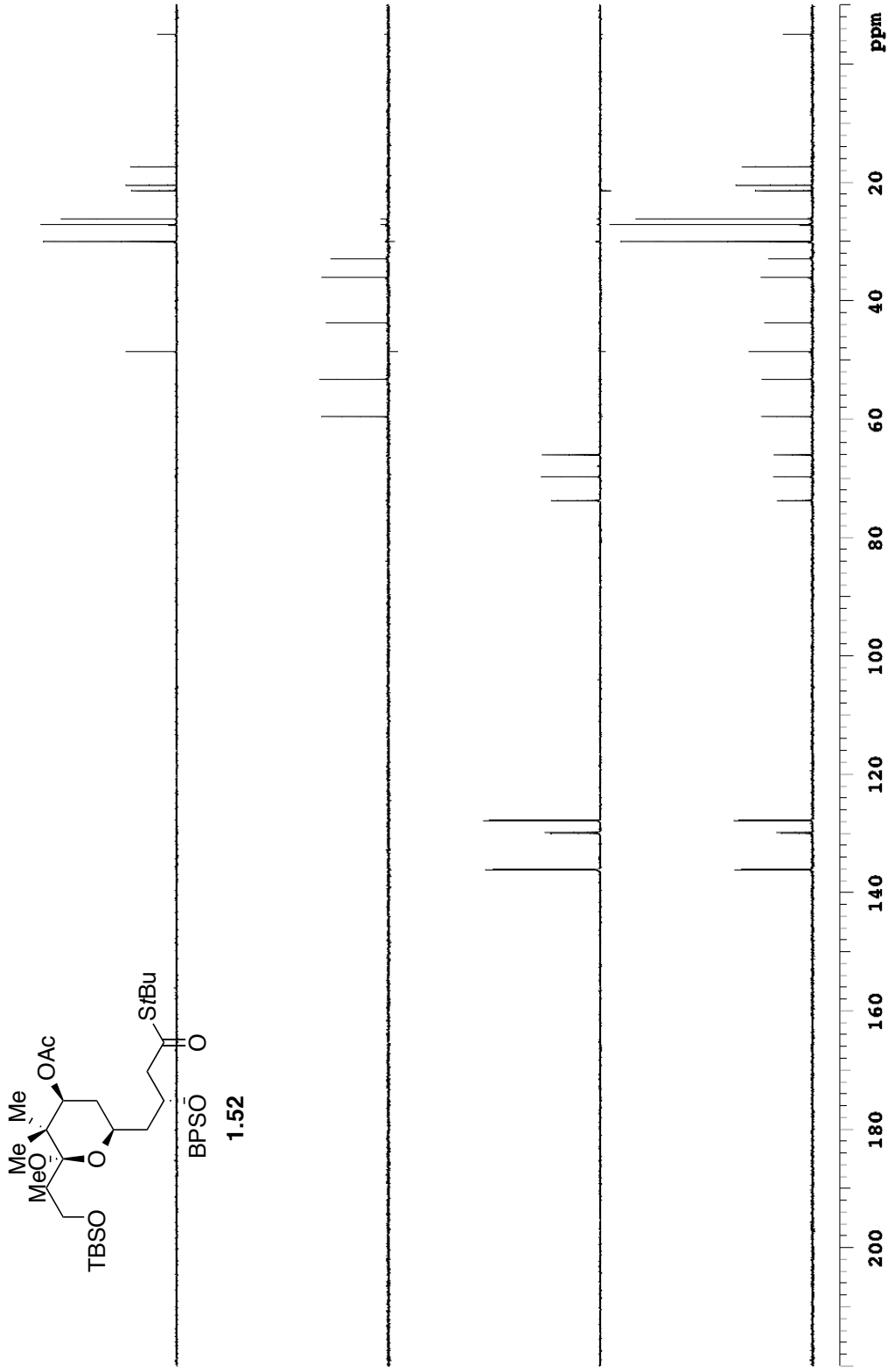


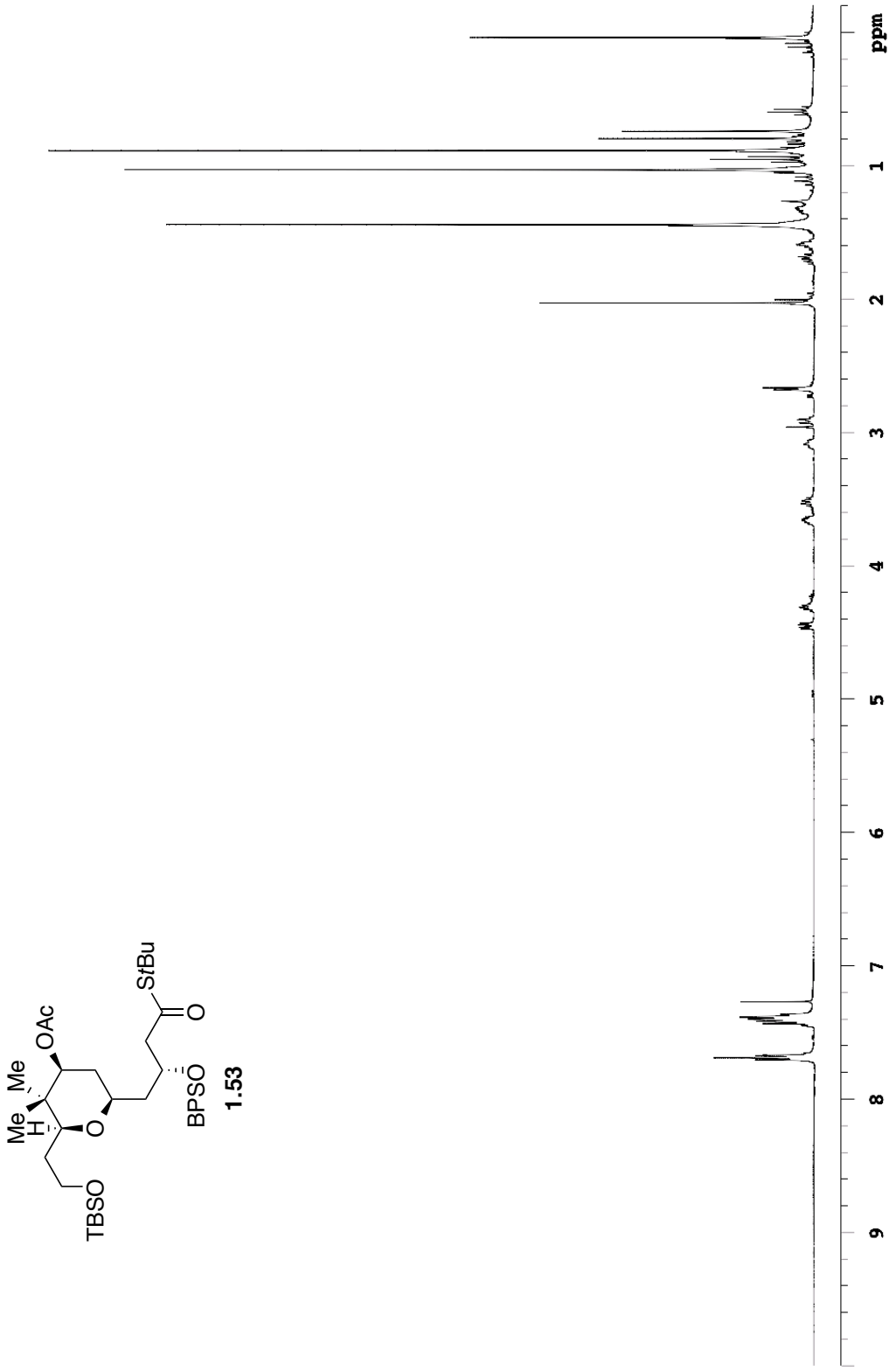
1.20

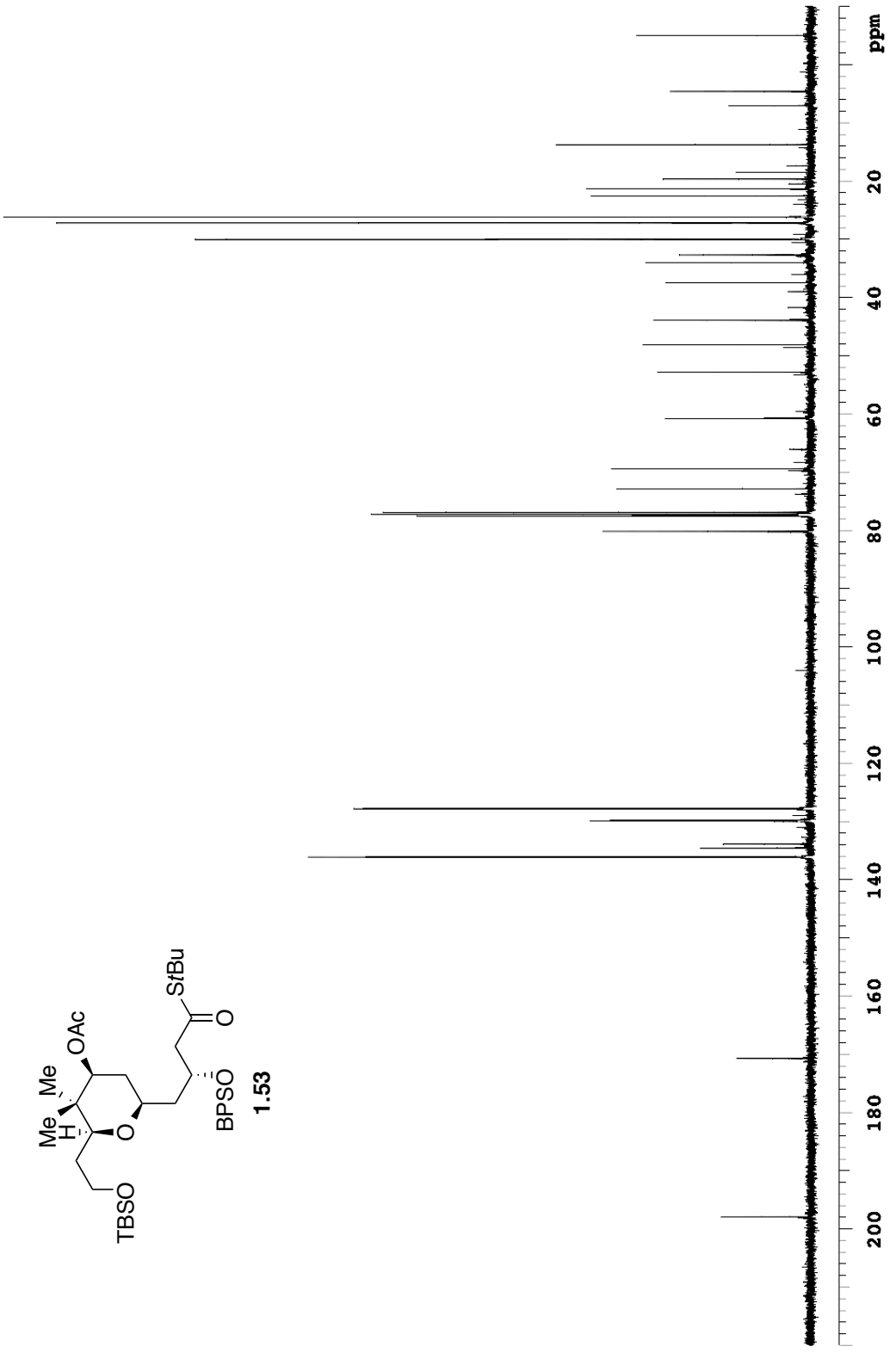

ppm
20
40
60
80
100
120
140
160
180
200
StBu
O
OAc
Me
Me
MeO
O
BPSO
H
O
1.20

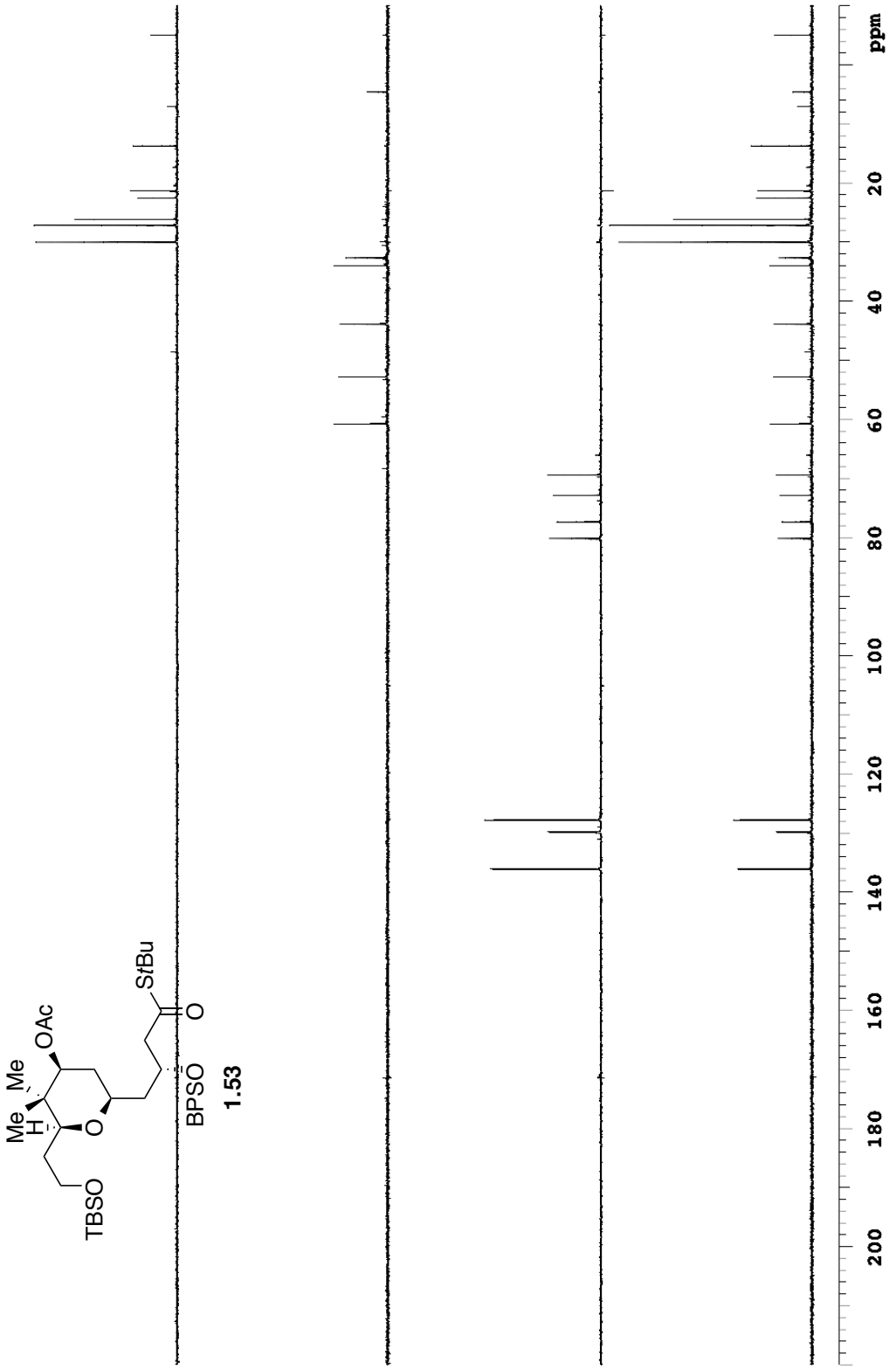

StBu
O
OAc
Me
Me
MeO
O
BPSO
TBSO
1.52
ppm
1
2
3
4
5
6
7
8
9

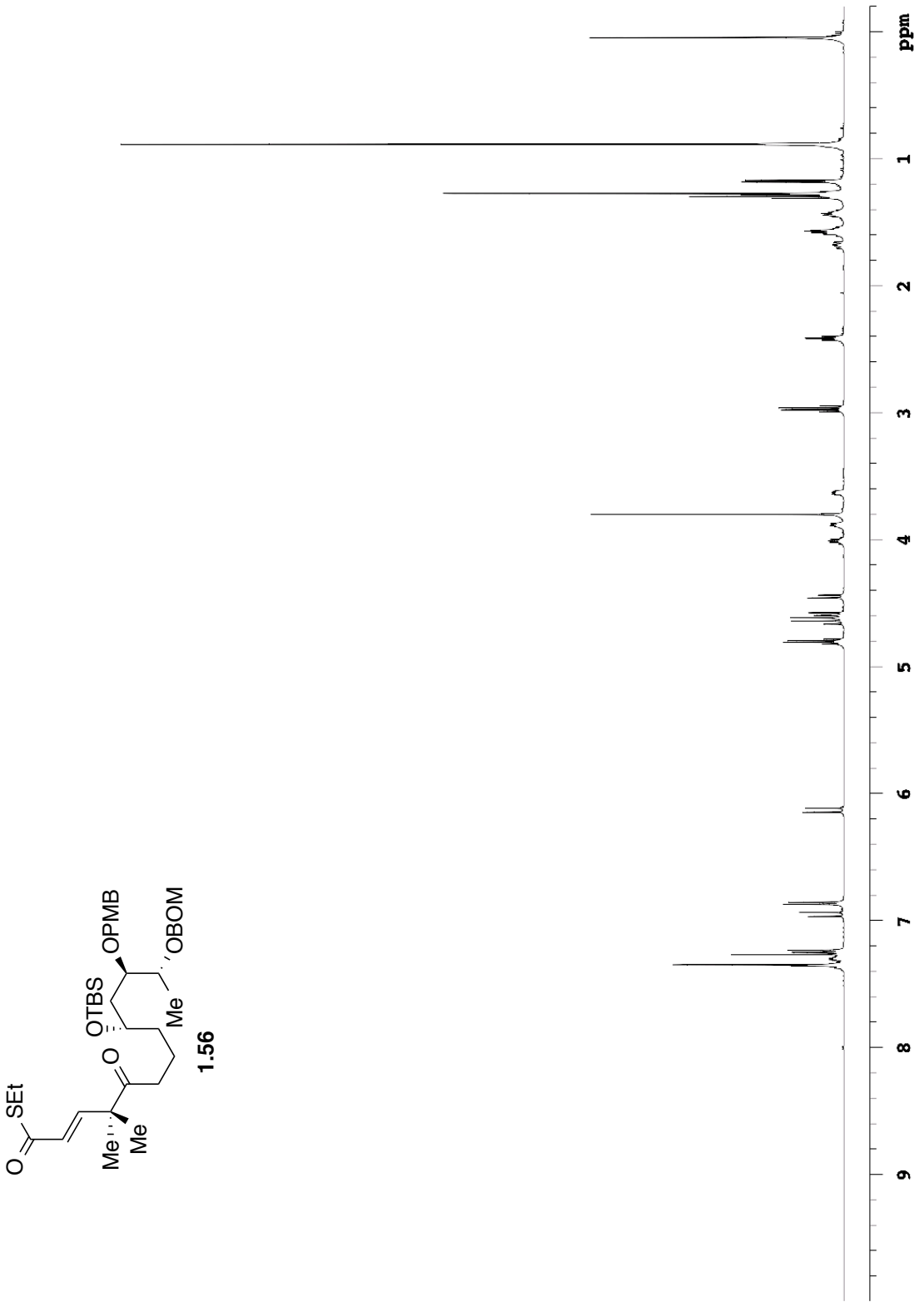

ppm
20
40
60
80
100
120
140
160
180
200
TBSO
MeO
Me
Me
OAc
O
BPSO
O
StBu
1.52

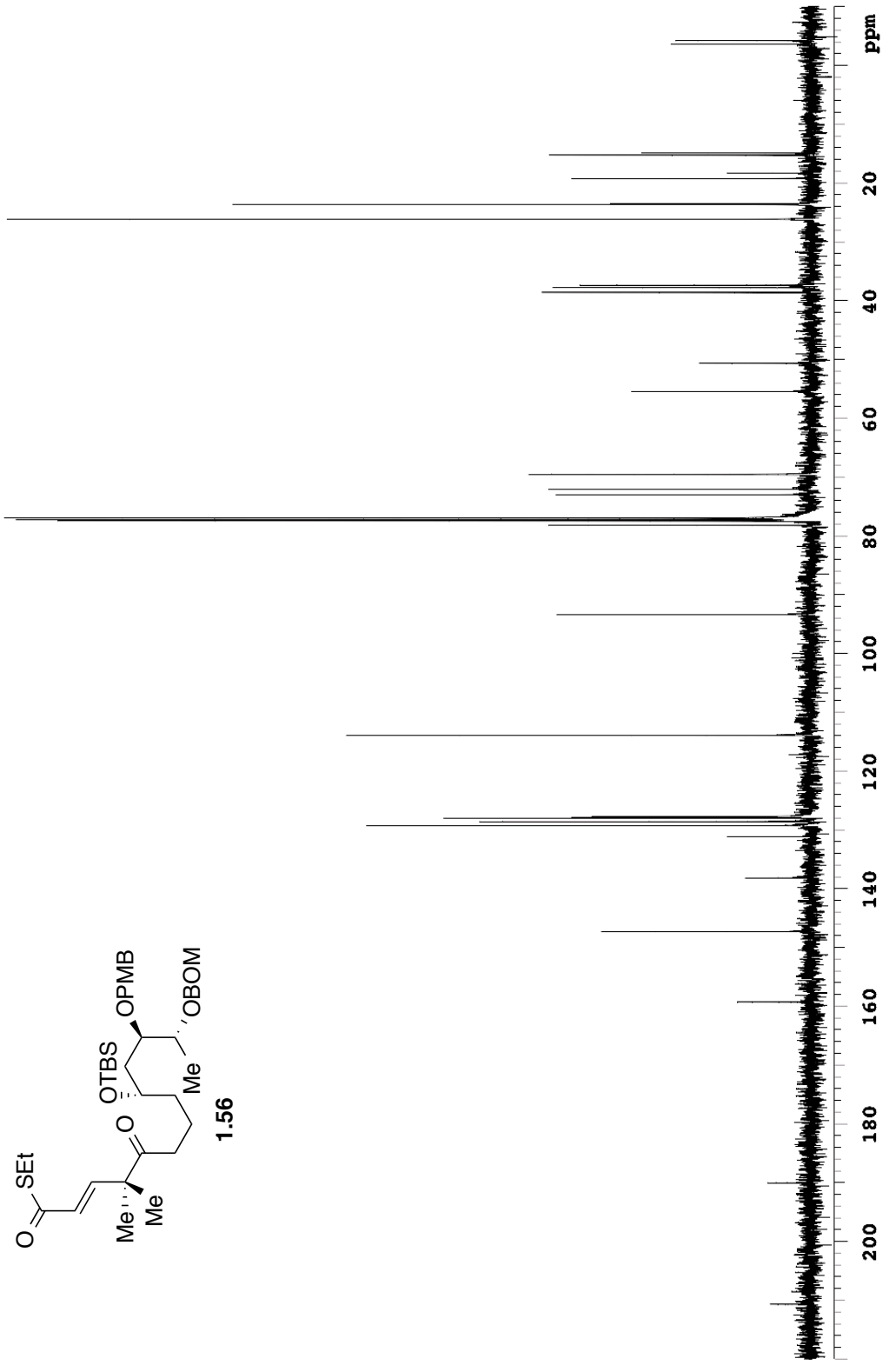

Me
Me
OAc
MeO
O
TBSO
StBu
O
BPSO
1.52
200
180
160
140
120
100
80
60
40
20
ppm

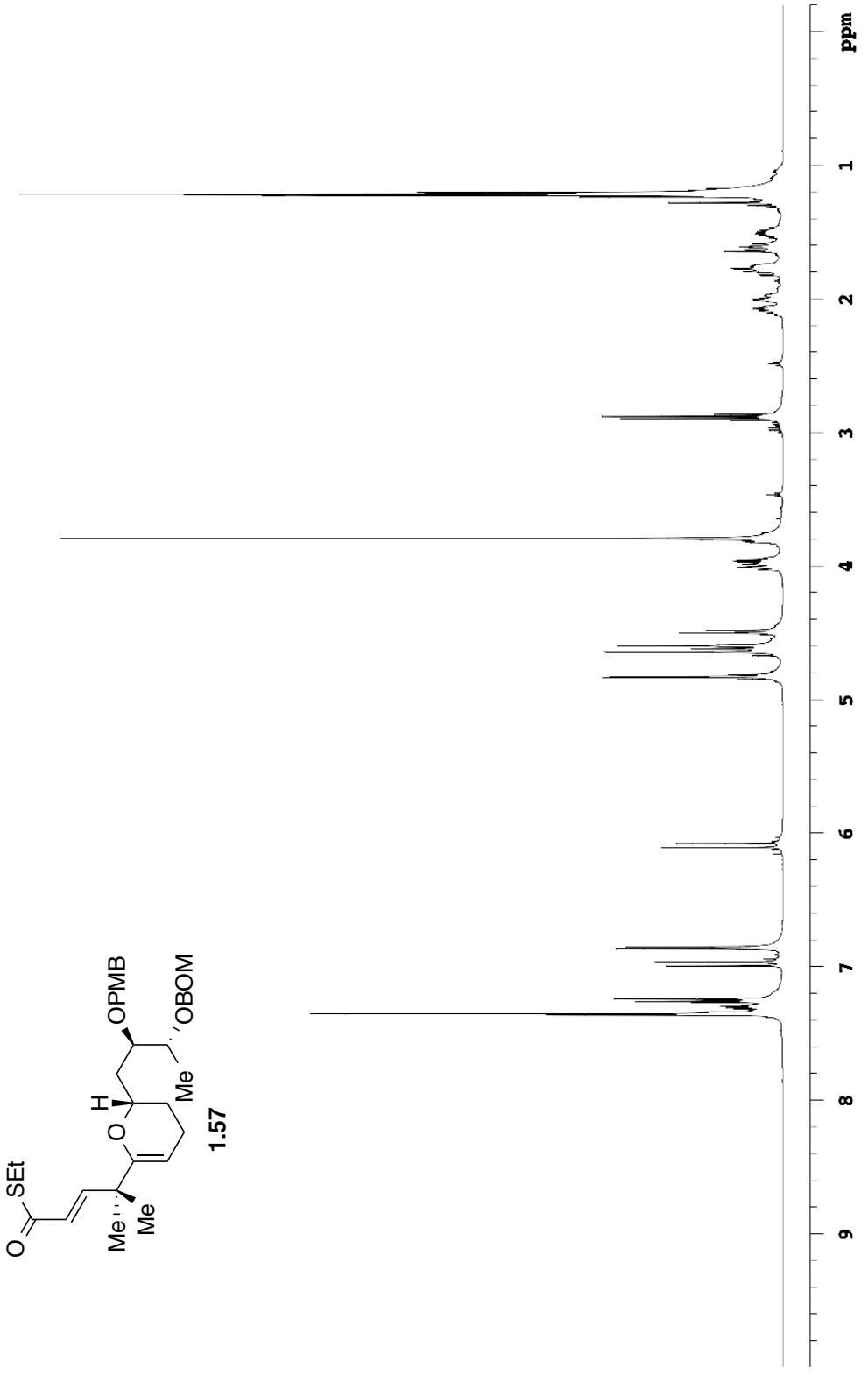

StBu
OAc
Me
Me
H
O
O
BPSO
TBSO
1.53
ppm
1
2
3
4
5
6
7
8
9

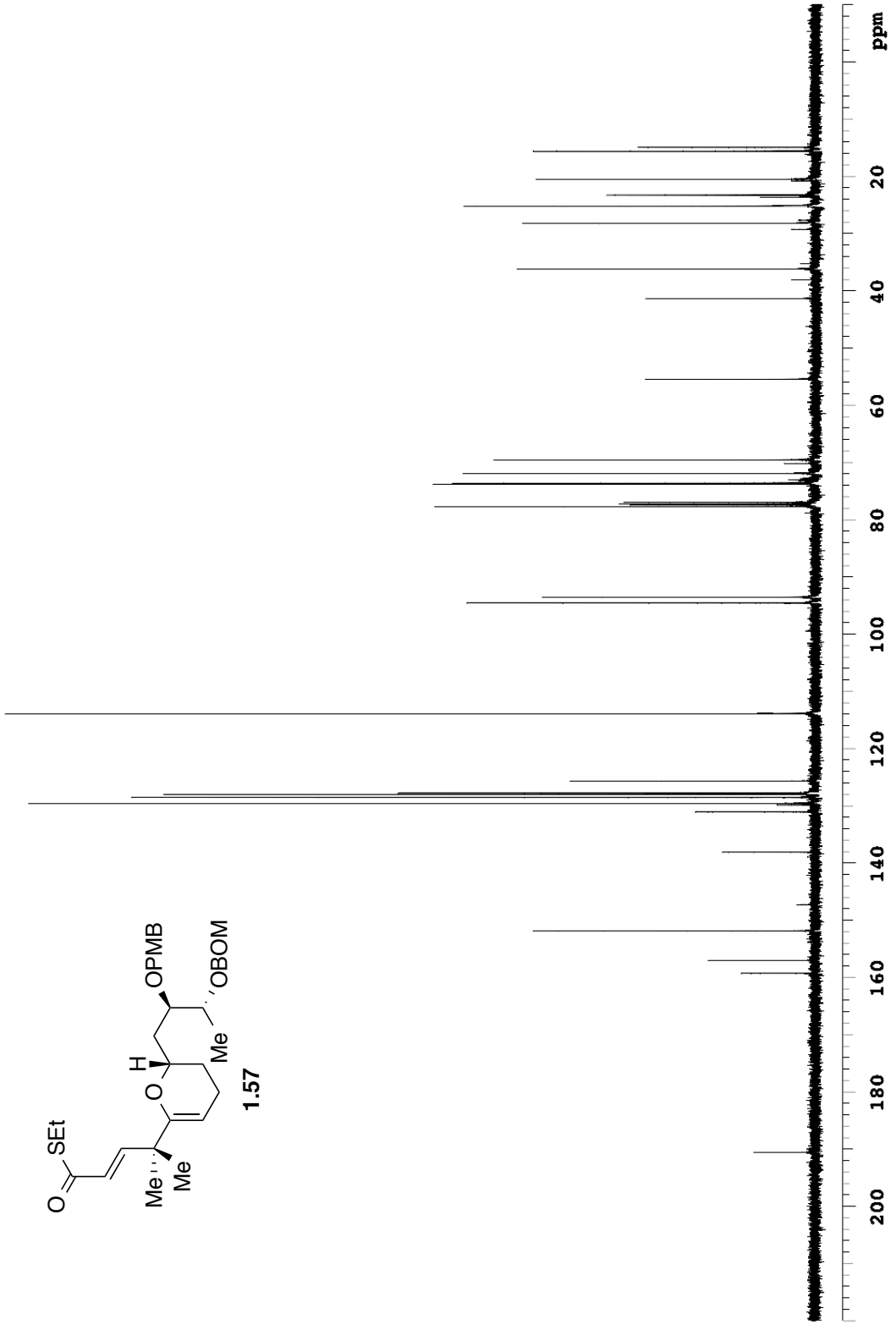

TBSO
Me
Me
H
OAc
O
BPSO
StBu
O
1.53
ppm
20
40
60
80
100
120
140
160
180
200

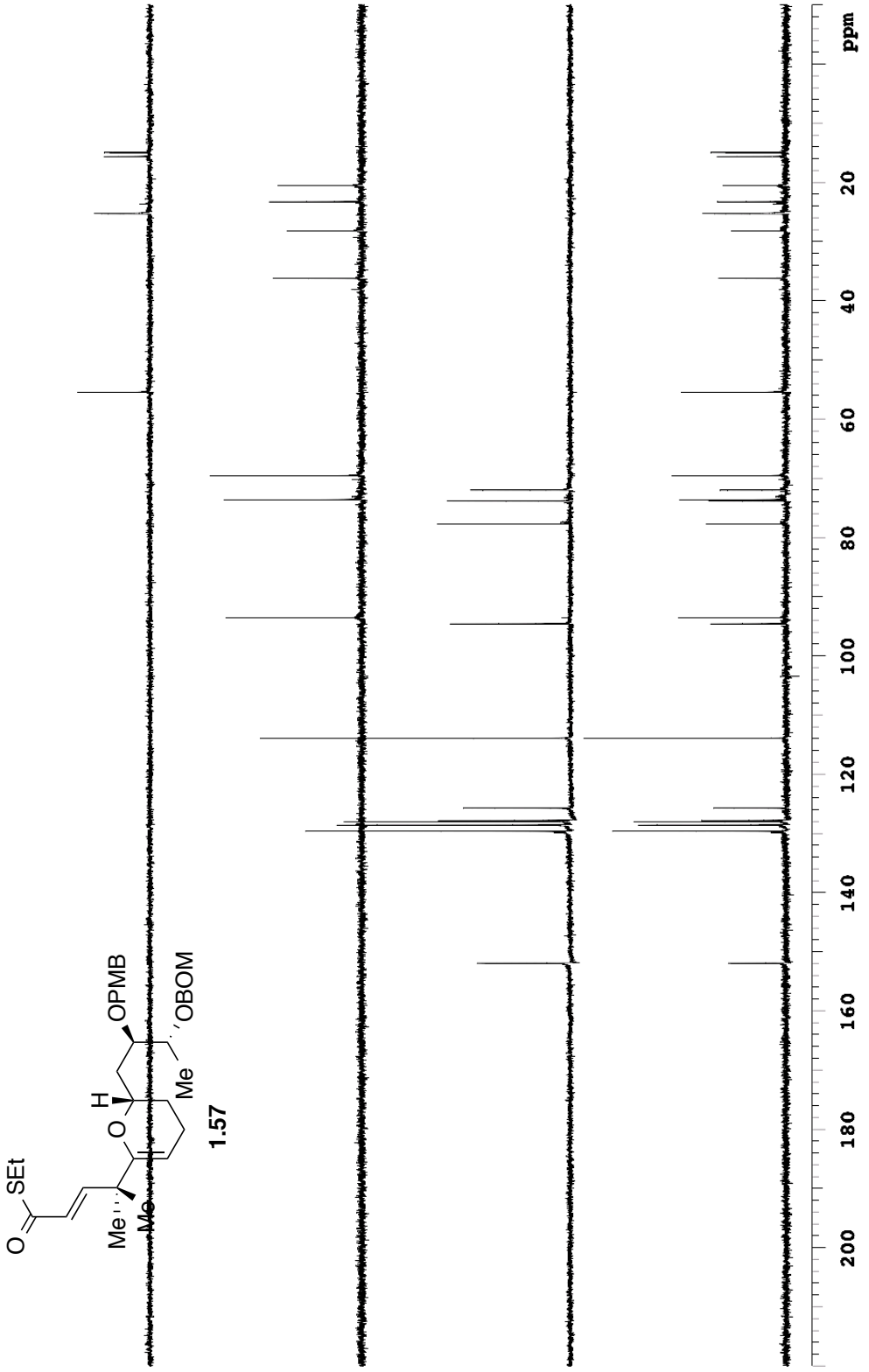

Me
Me
H
OAc
O
TBSO
BPSO
O
StBu
1.53
ppm
200
180
160
140
120
100
80
60
40
20

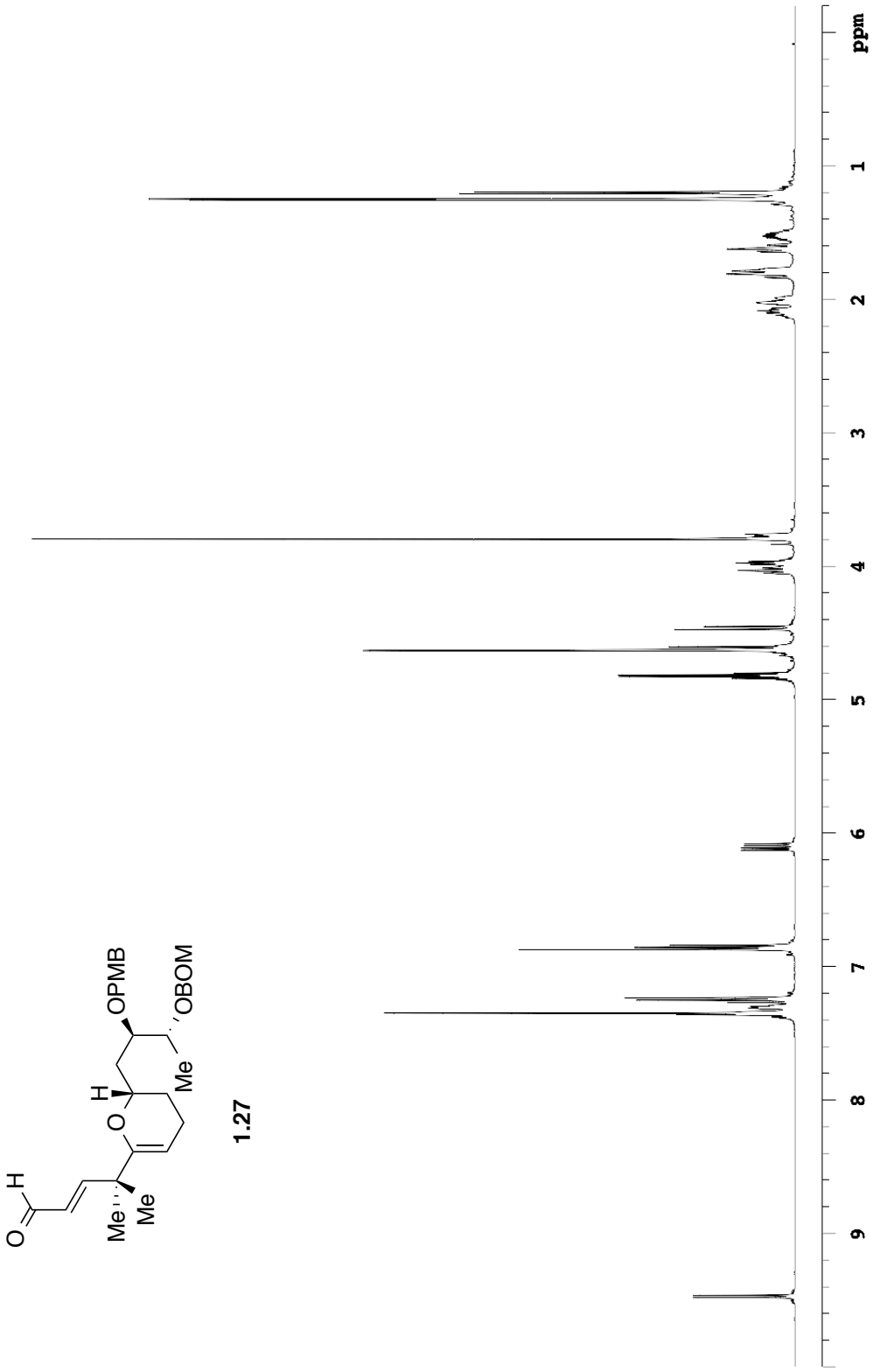

O
SEt
Me
Me
O
OTBS
OPMB
Me
OBOM
1.56
ppm
9
8
7
6
5
4
3
2
1

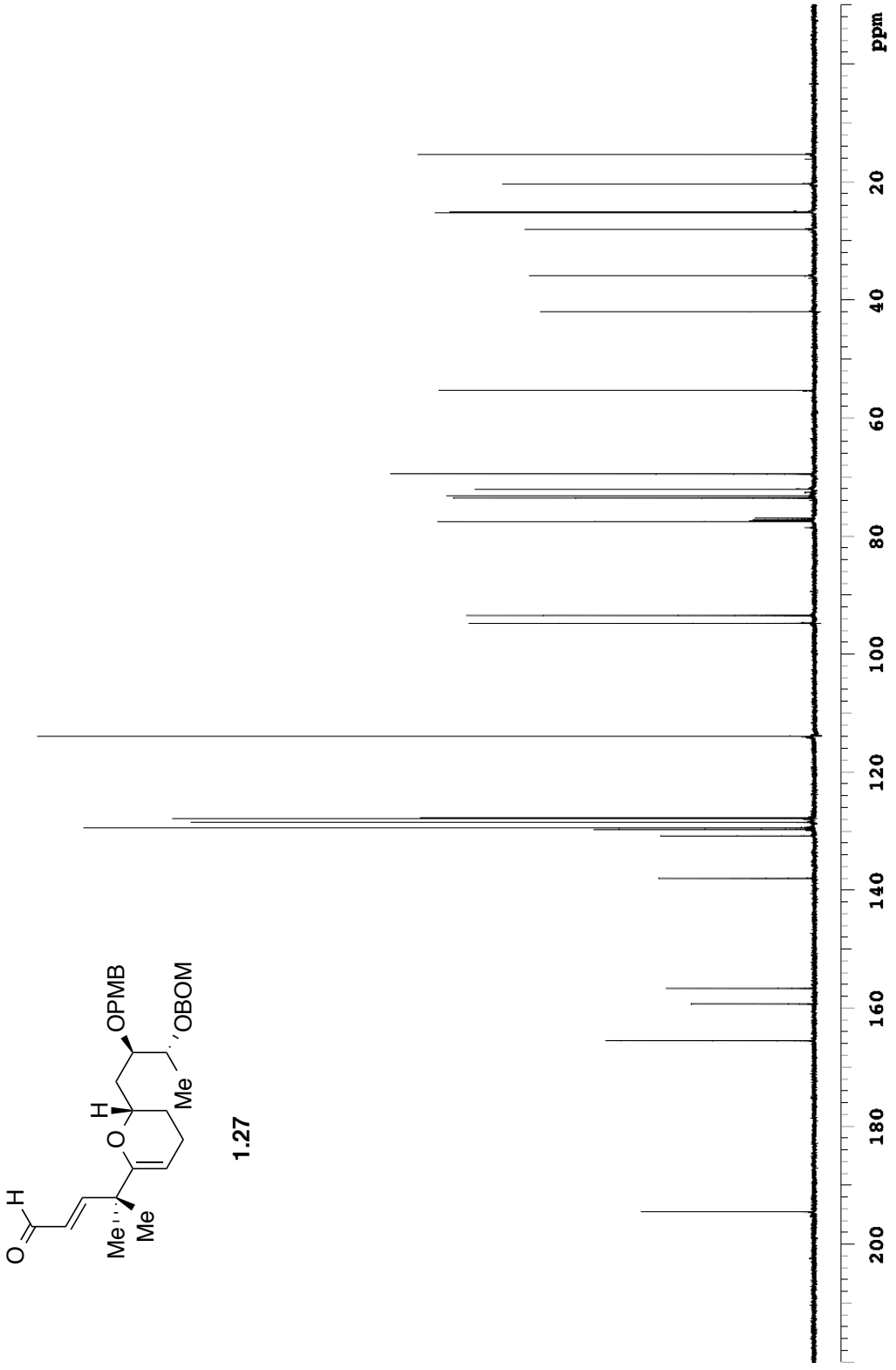

O
SEt
Me
Me
O
OTBS
OPMB
Me
OBOM
1.56
ppm
200
180
160
140
120
100
80
60
40
20

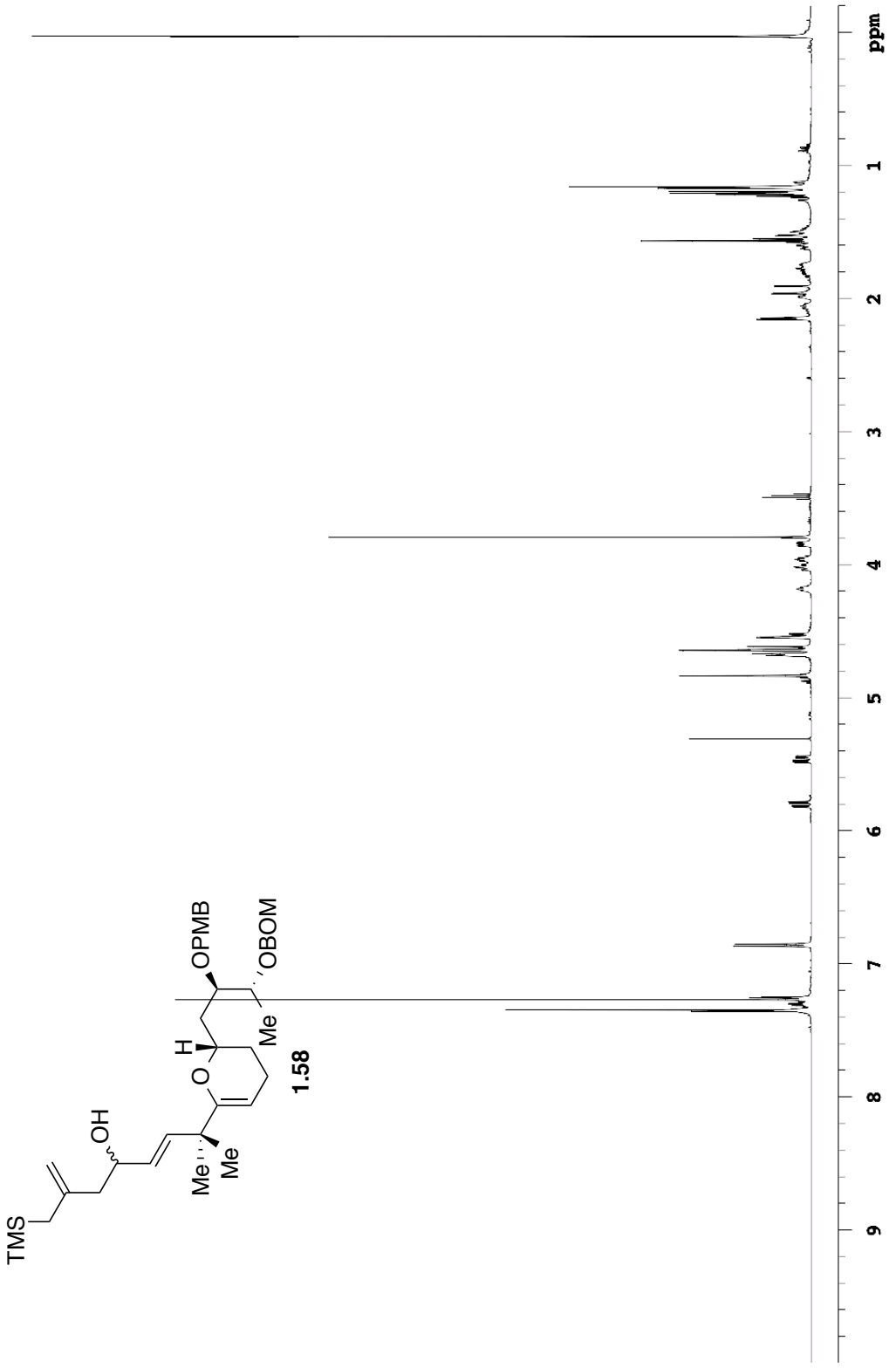

O
SEt
Me
Me
H
O
OPMB
Me
OBOM
1.57
ppm
1
2
3
4
5
6
7
8
9

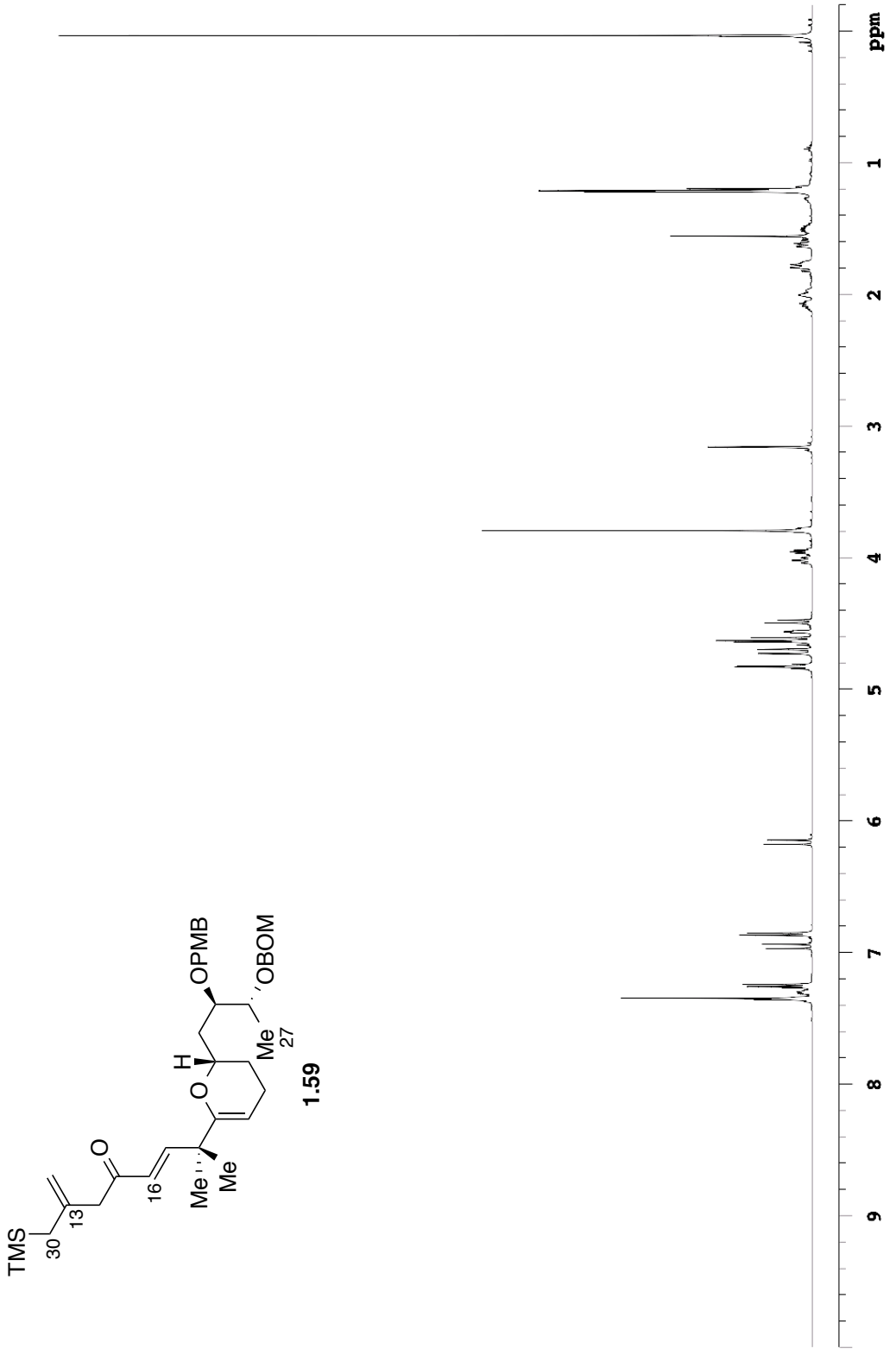

O
SEt
Me
Me
O
H
OPMB
Me
OBOM
1.57
200
180
160
140
120
100
80
60
40
20
ppm

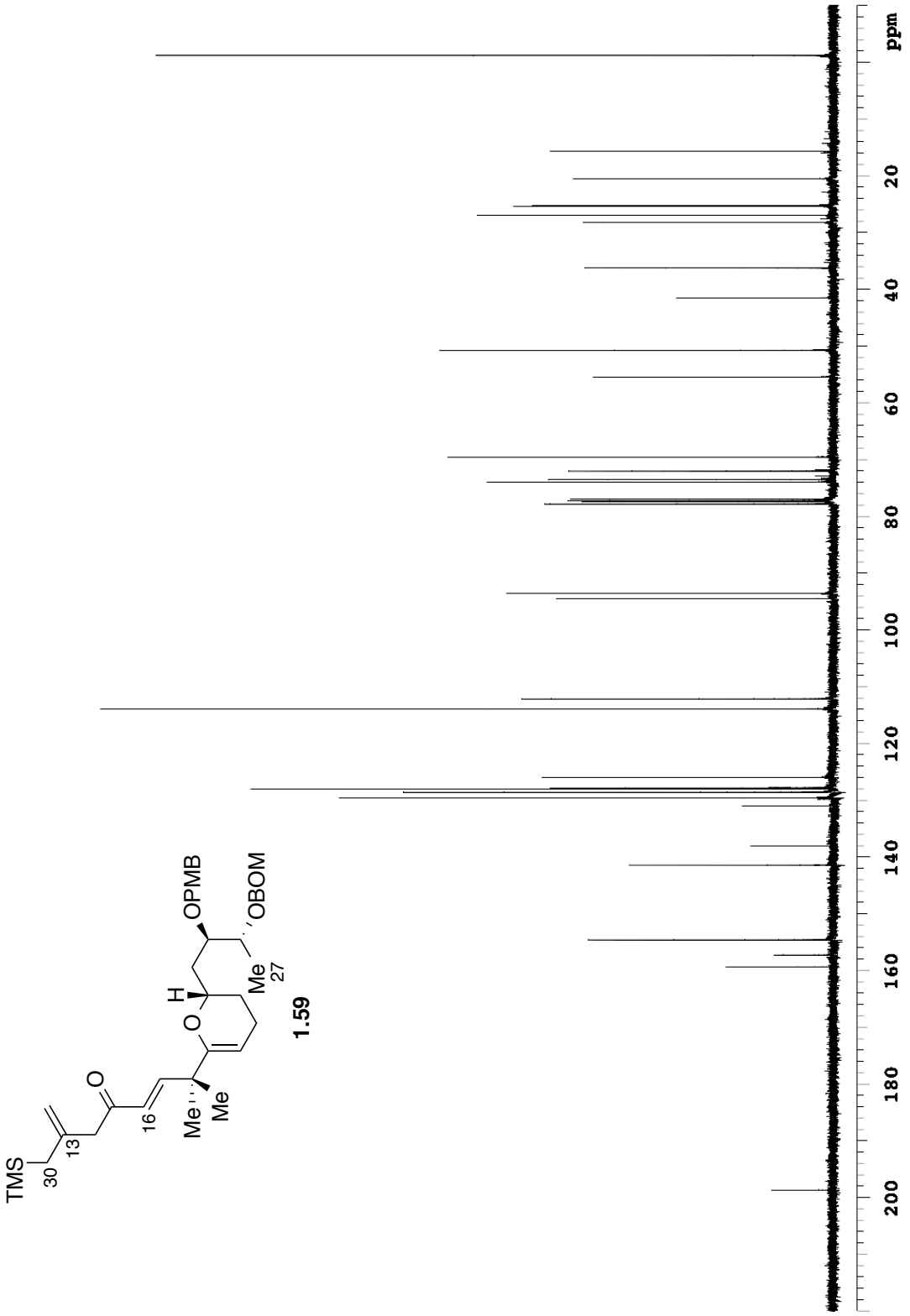

O
SEt
Me
Me
O
H
OPMB
Me
OBOM
1.57
200
180
160
140
120
100
80
60
40
20
ppm

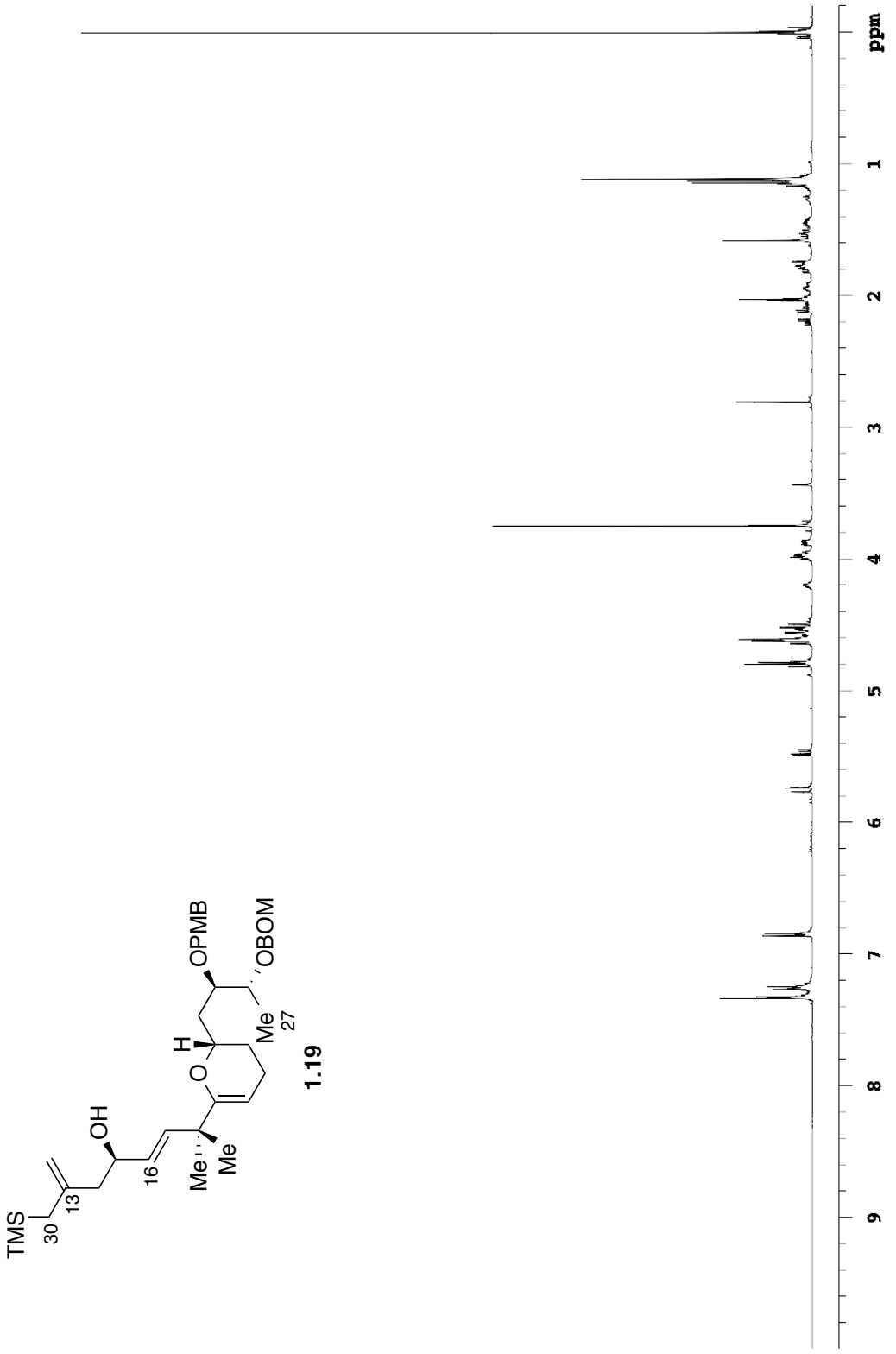

OPMB
OBOM
Me
Me
Me
H
O
O
H
1.27
ppm
1
2
3
4
5
6
7
8
9

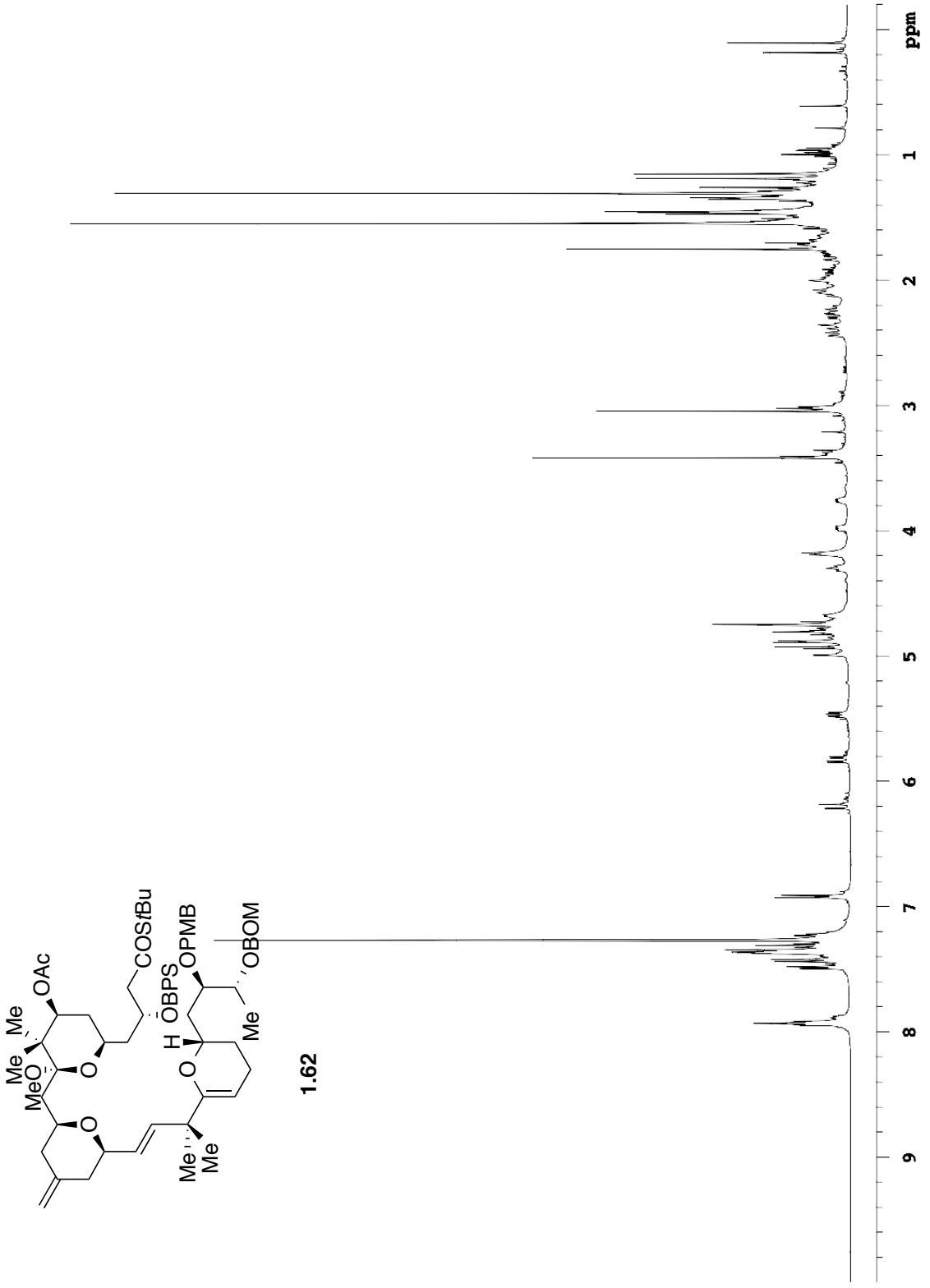

OPMB
OBOM
Me
Me
Me
H
O
O
H
1.27
ppm
20
40
60
80
100
120
140
160
180
200


TMS
OH
Me
Me
O
H
Me
OPMB
OBOM
1.58
ppm
1
2
3
4
5
6
7
8
9


TMS
30
13
O
16
Me
Me
O
H
OPMB
OBOM
Me
27
1.59
ppm
1
2
3
4
5
6
7
8
9

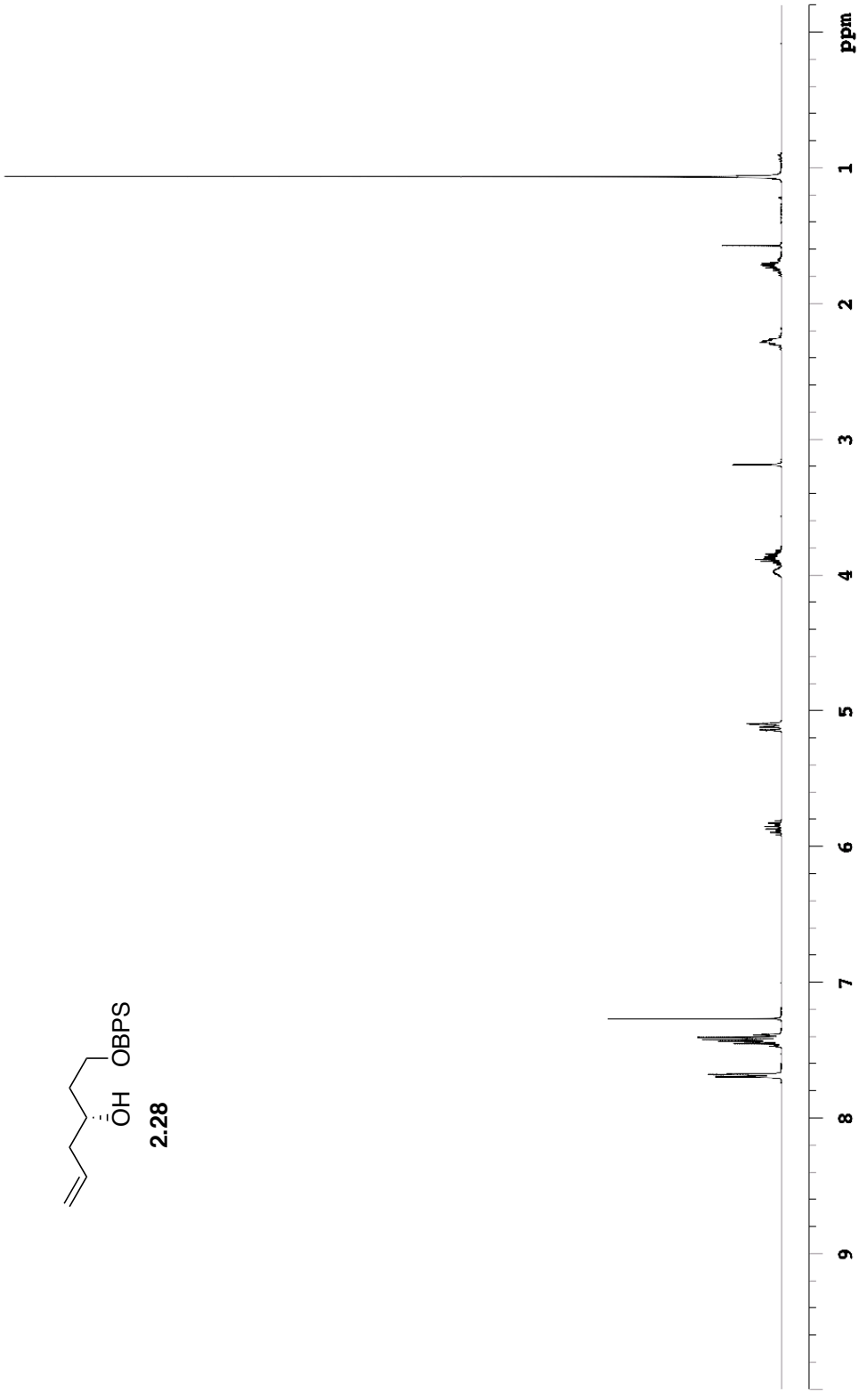

TMS
30
13
O
16
Me
Me
O
H
OPMB
OBOM
Me
27
1.59
ppm
20
40
60
80
100
120
140
160
180
200


TMS
30
13
OH
16
Me
Me
O
H
OPMB
OBOM
Me
27
1.19
ppm
1
2
3
4
5
6
7
8
9

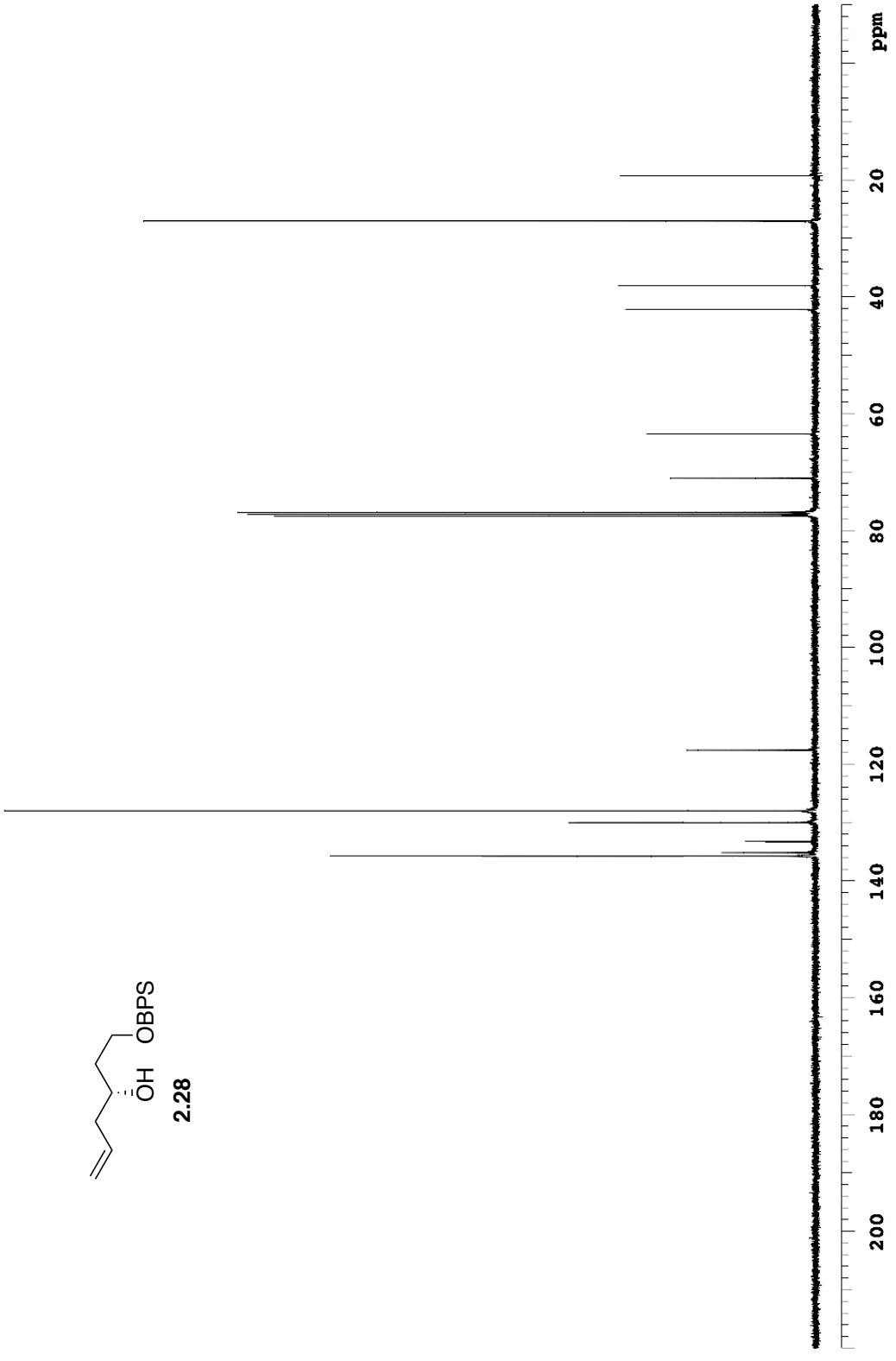

Me
Me
MeO
OAc
O
O
O
COStBu
H
OBPS
O
OPMB
Me
OBOM
Me
Me
1.62
ppm
9
8
7
6
5
4
3
2
1

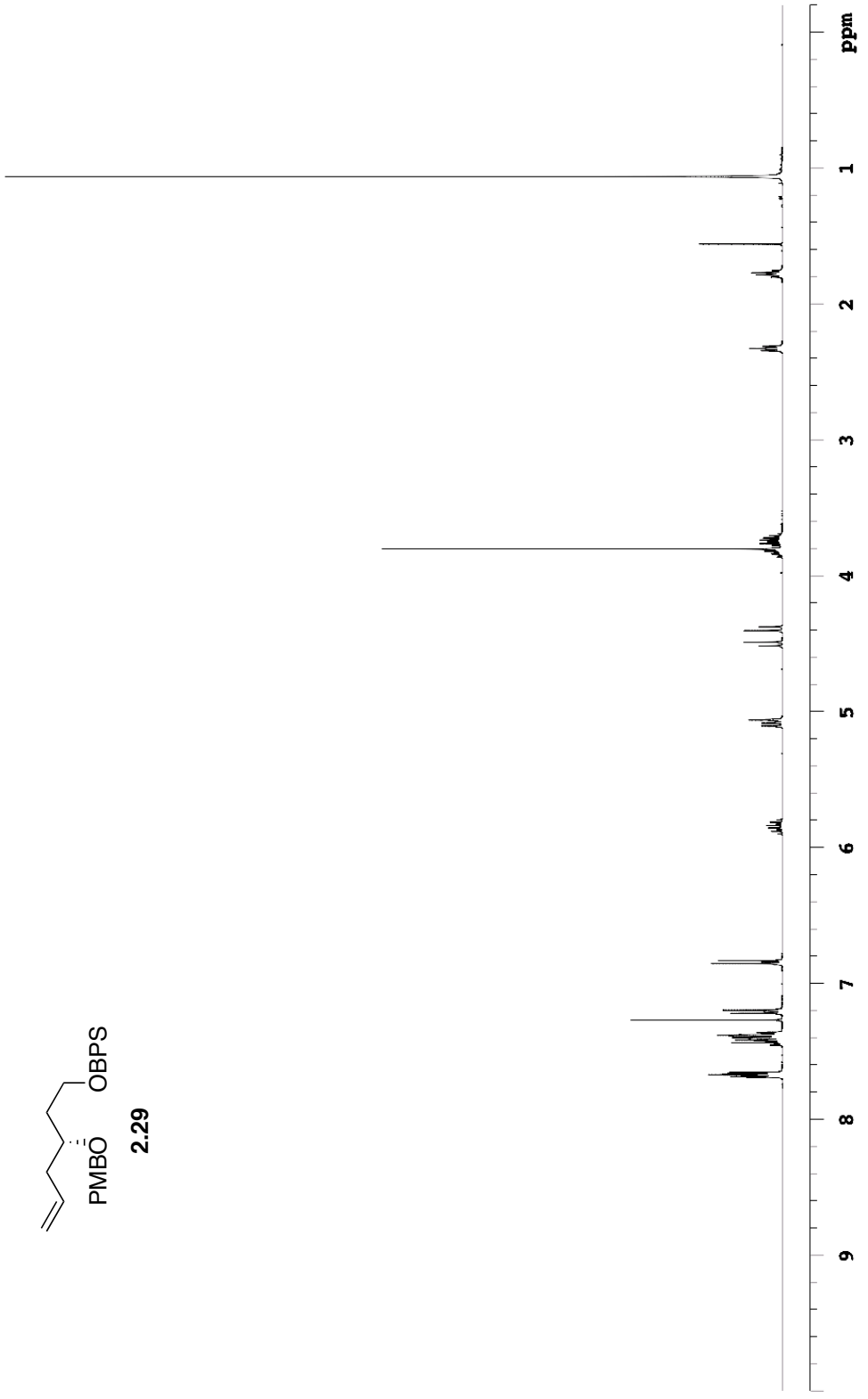
APPENDIX B

^{1}H AND ^{13}C NMR SPECTRA FOR CHAPTER 2

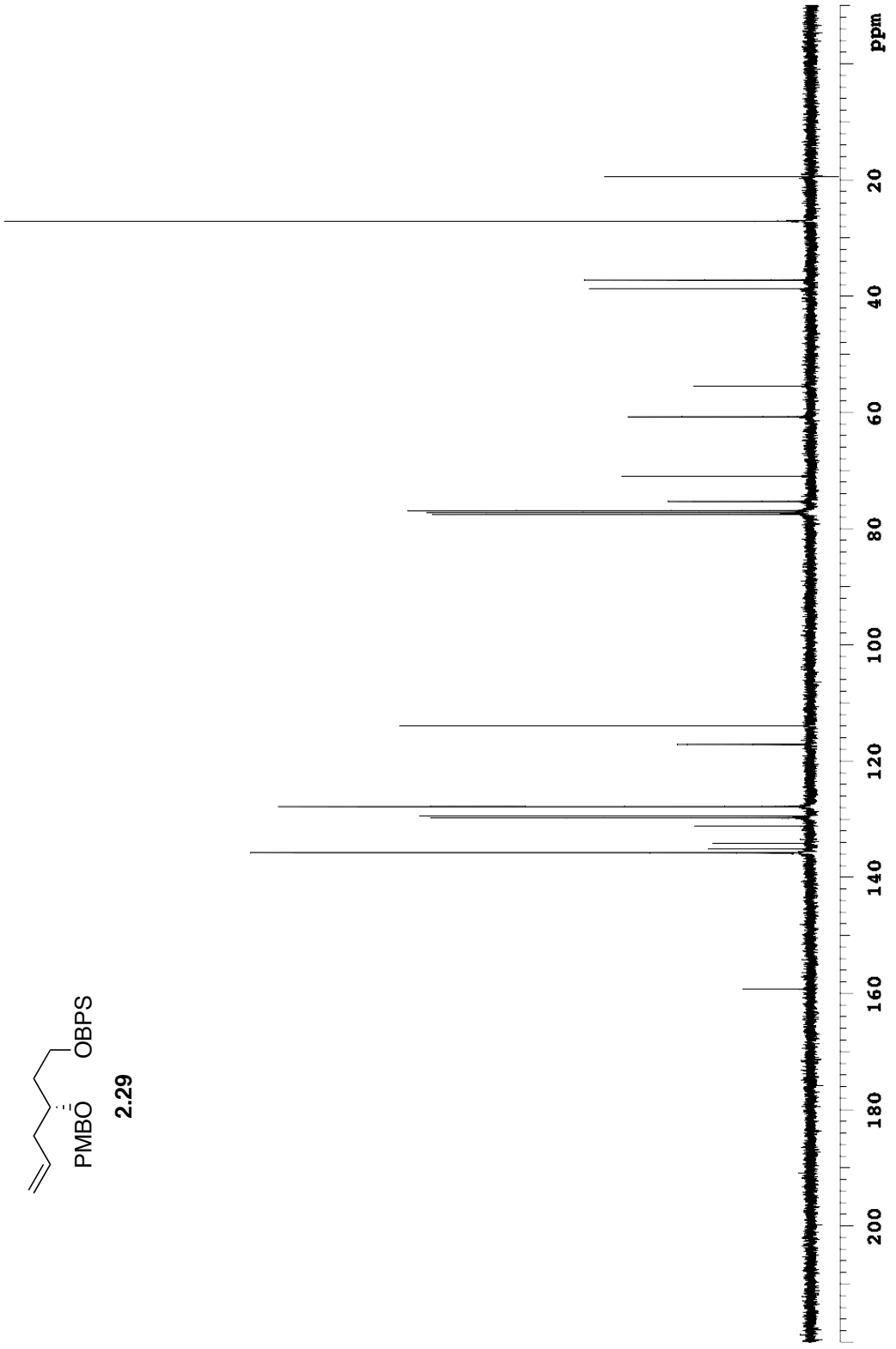


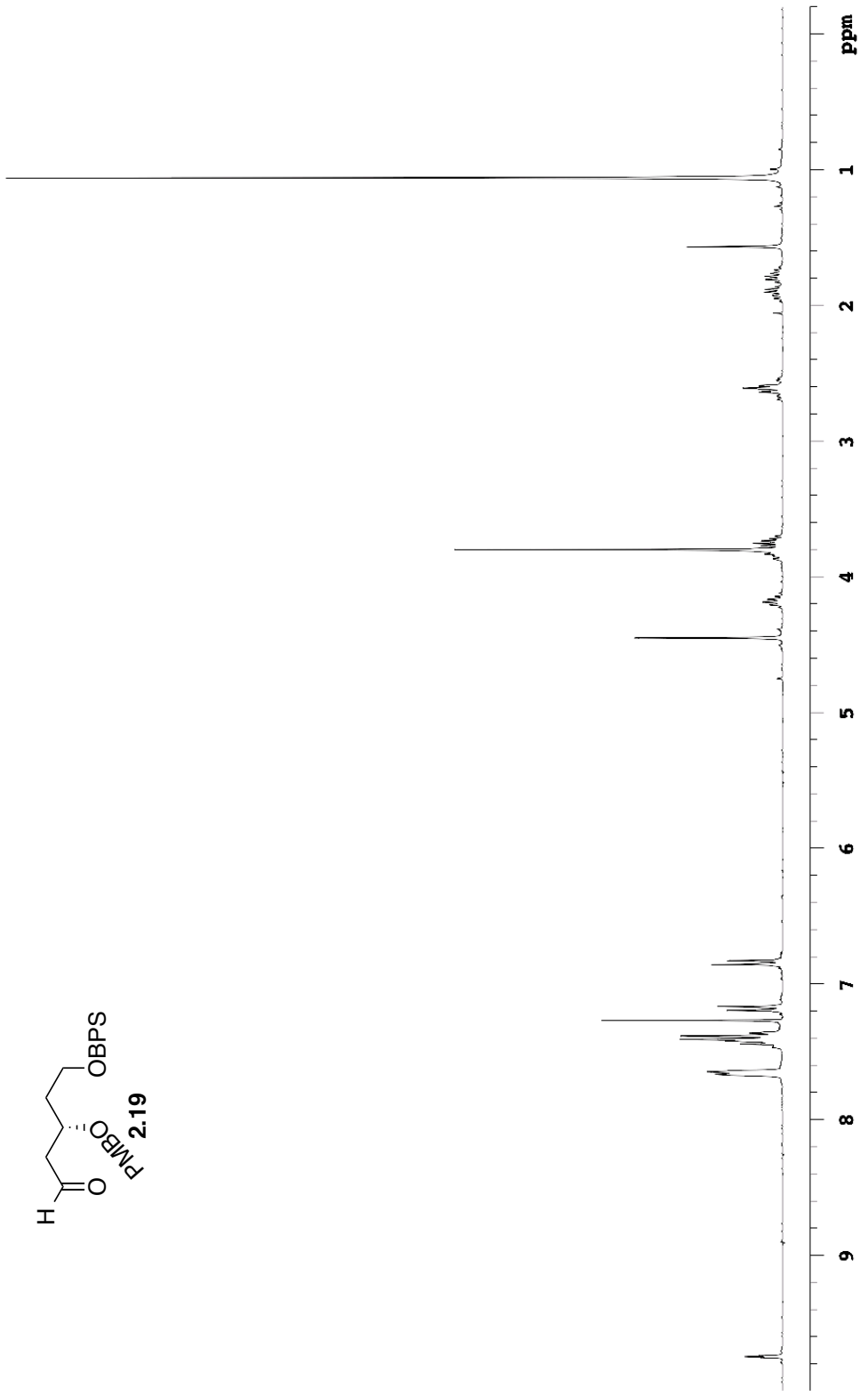
2.28

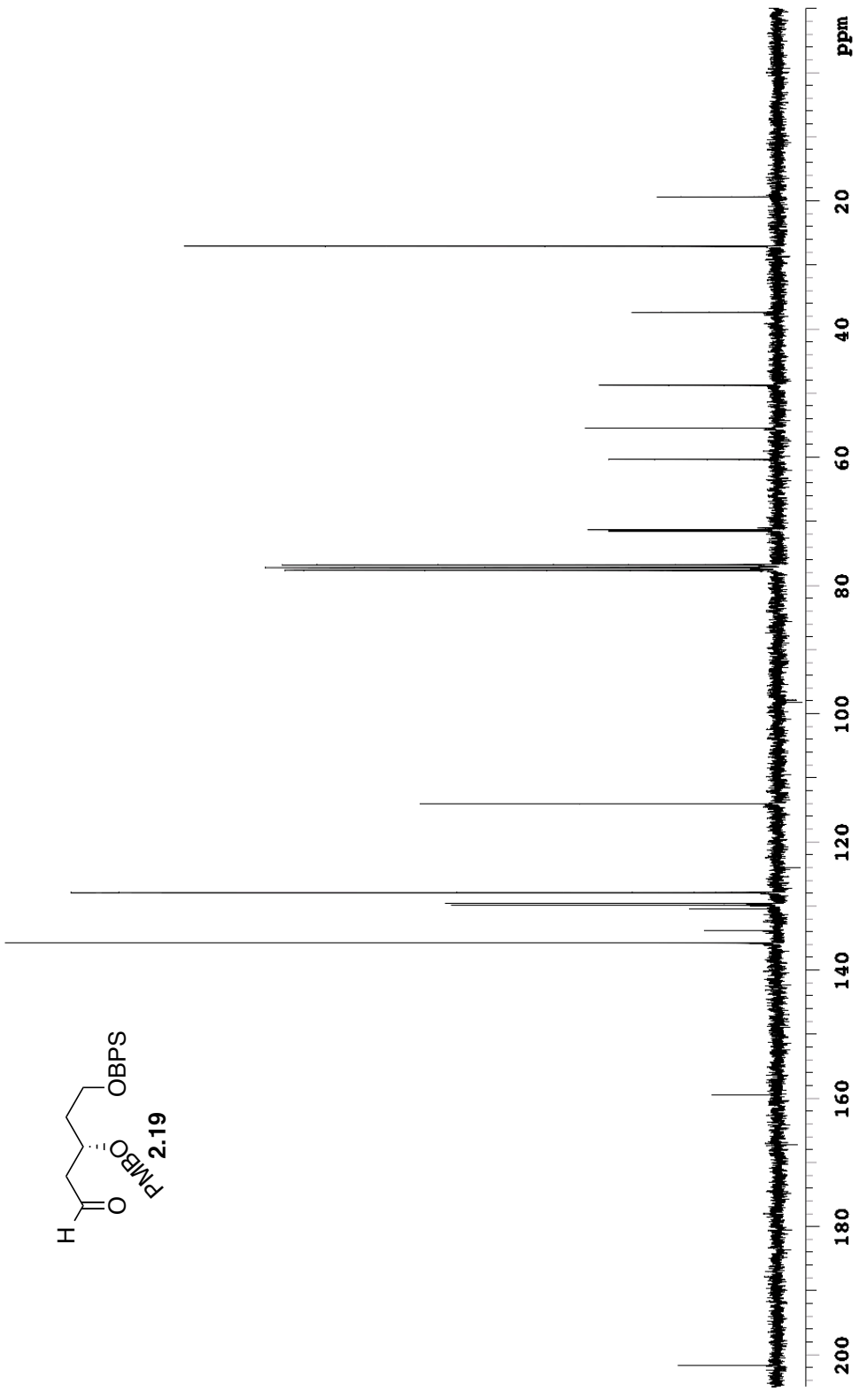

OBPS
OH
2.28
ppm
200
180
160
140
120
100
80
60
40
20

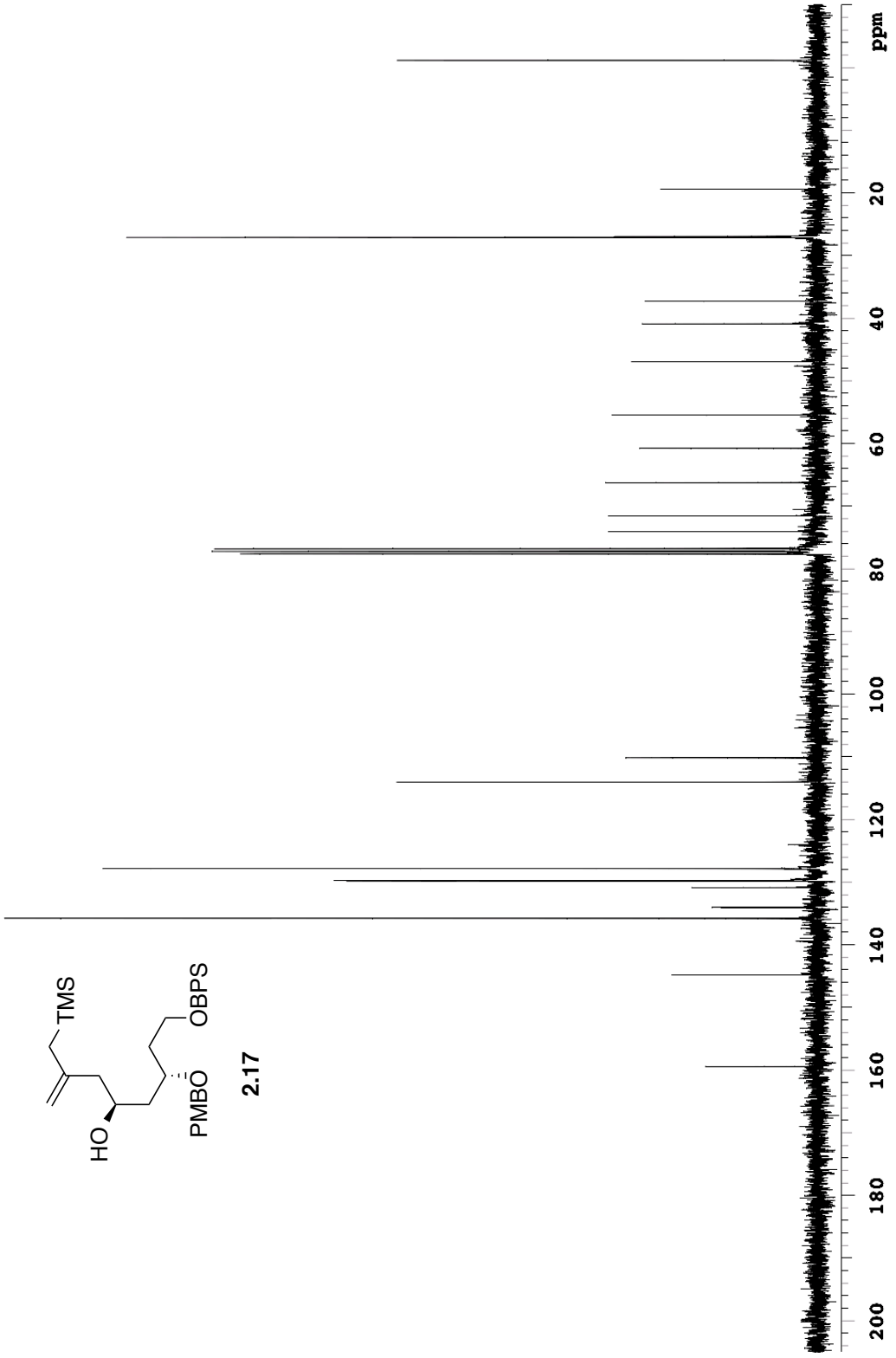


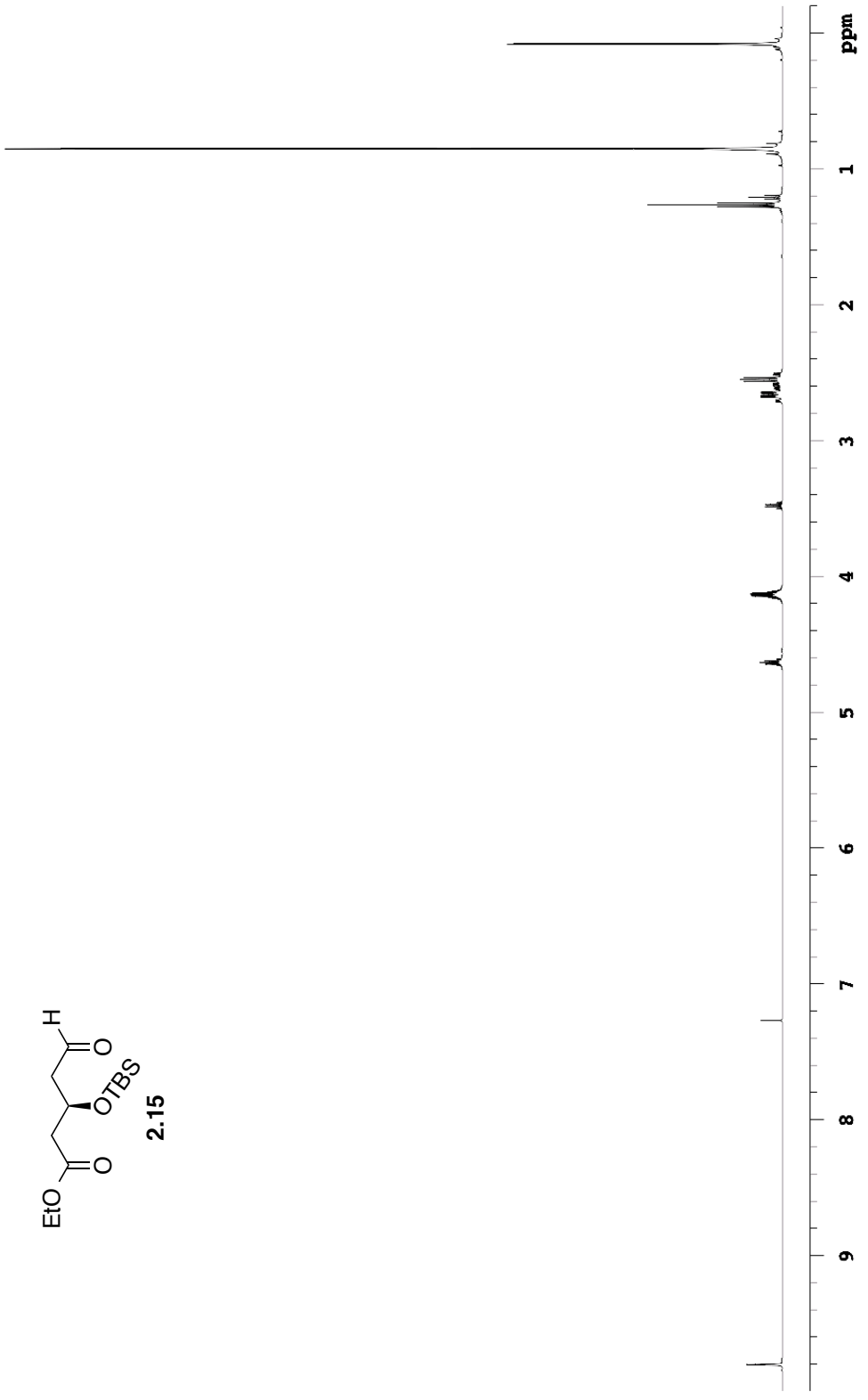
2.29

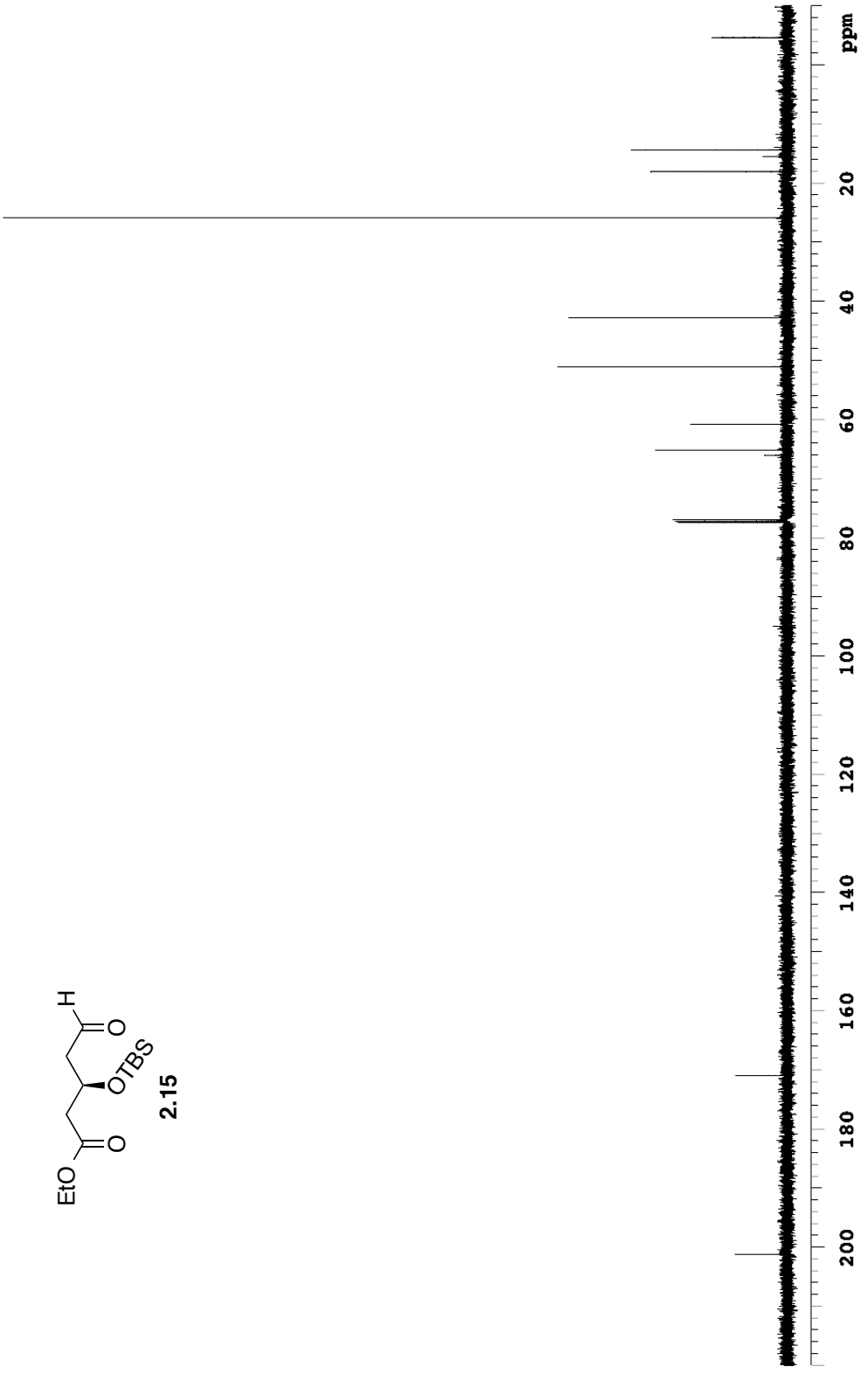

PMBO
OBPS
2.29
ppm
20
40
60
80
100
120
140
160
180
200

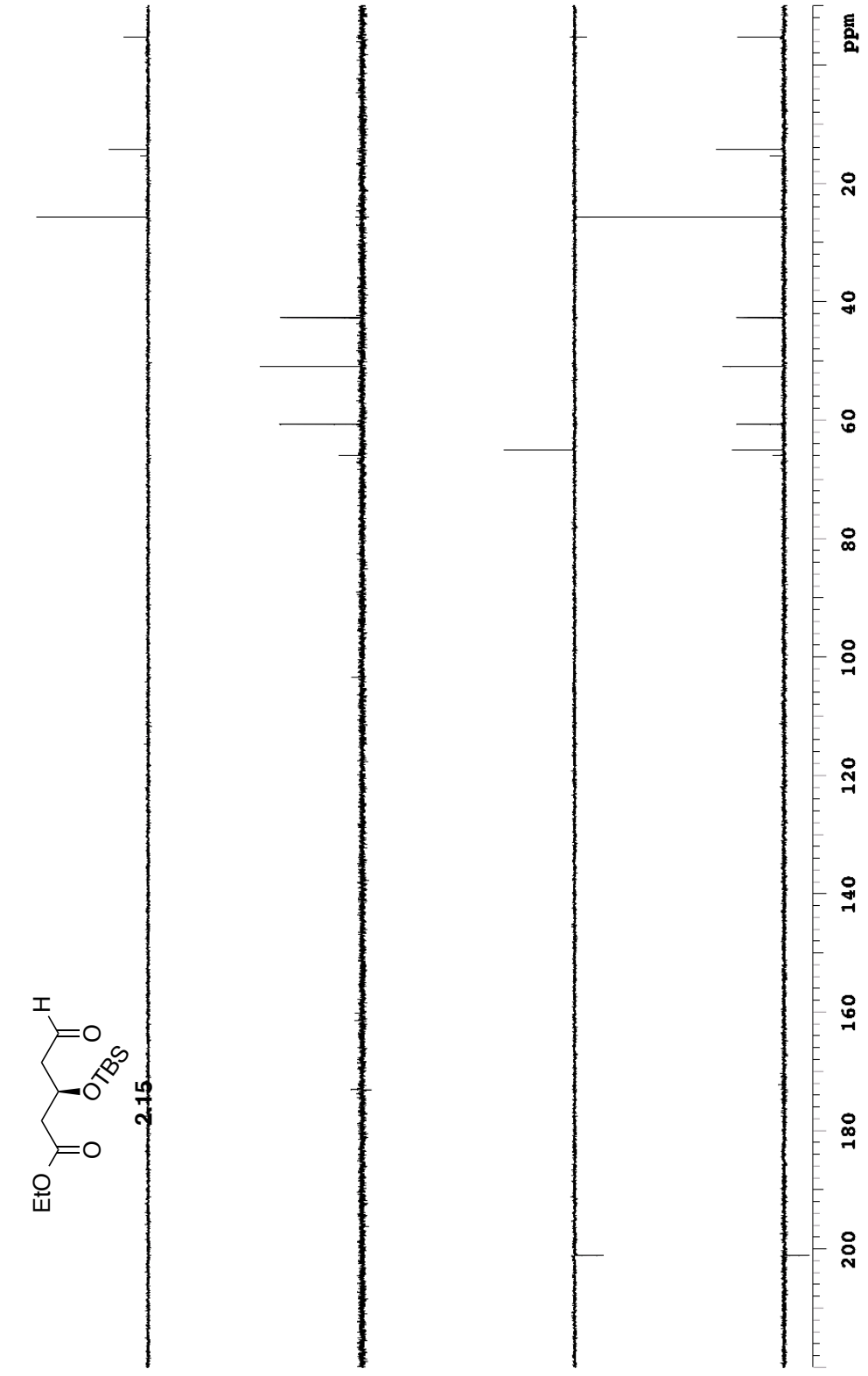

OBPS
PMBO
O
H
2.19
ppm
1
2
3
4
5
6
7
8
9

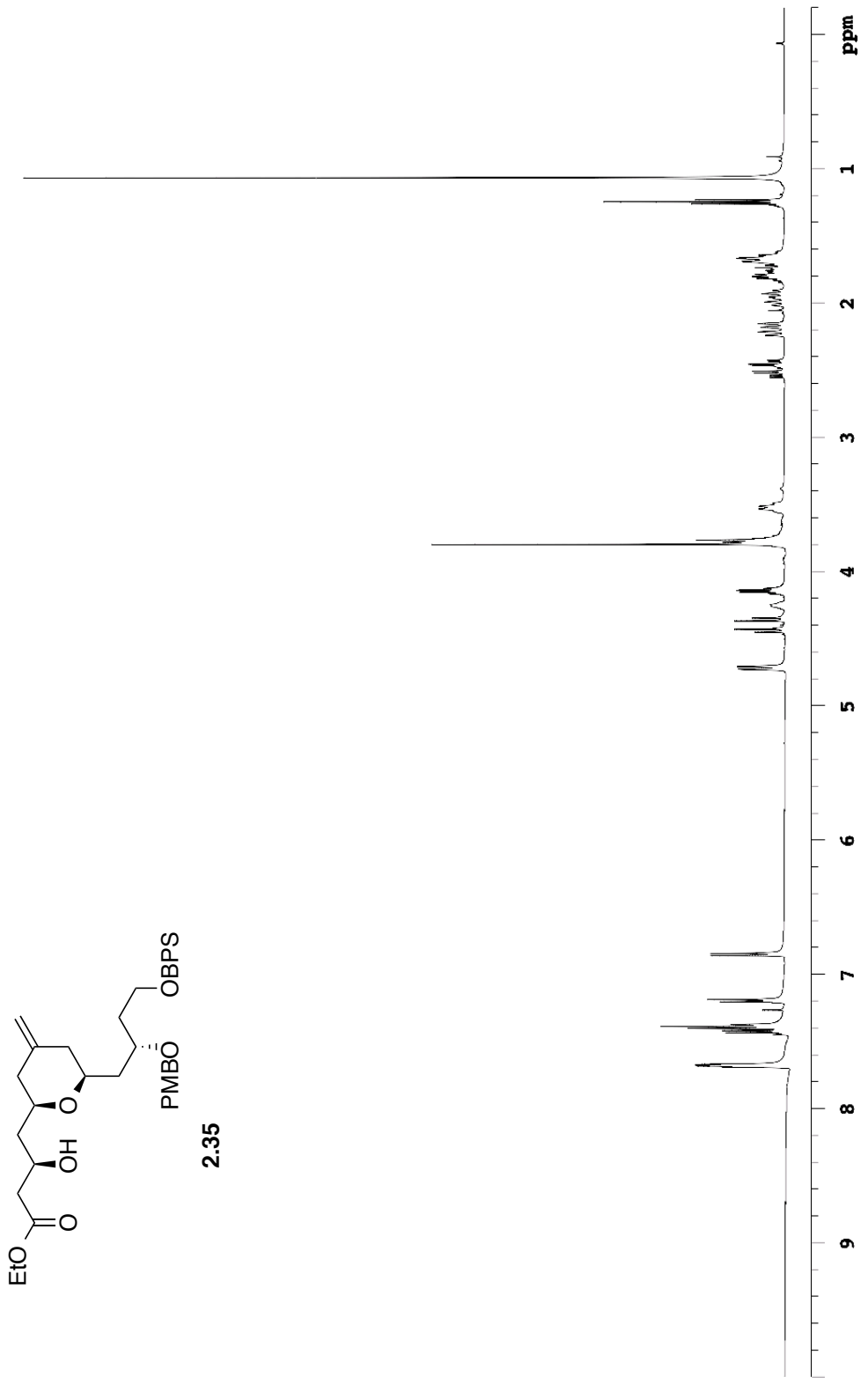

OBPS
PMBO
O
H
2.19
ppm
200
180
160
140
120
100
80
60
40
20


TMS
OBPS
HO
PMBO
2.17
ppm
200
180
160
140
120
100
80
60
40
20

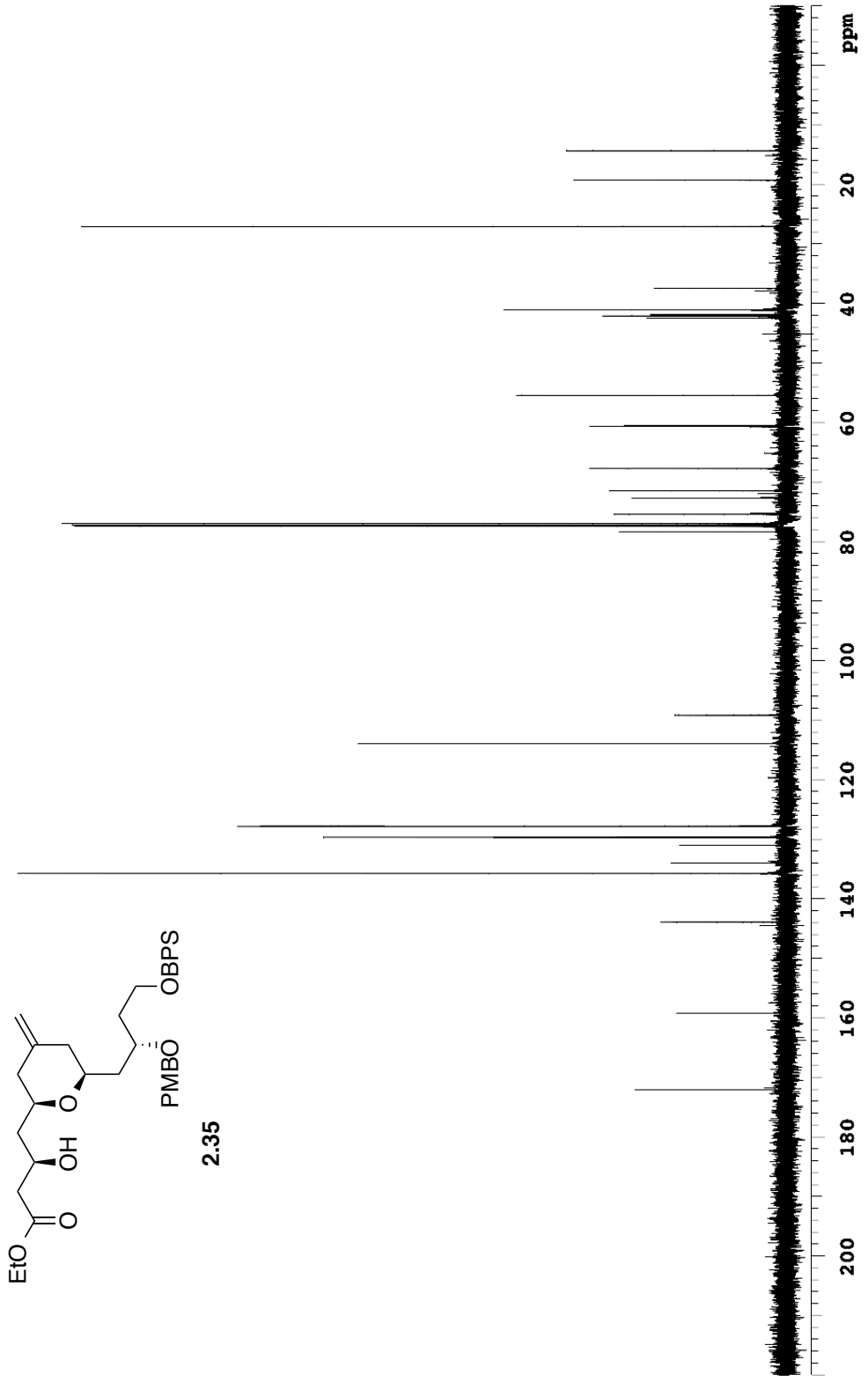

H
O
OTBS
O
EtO
2.15
ppm
1
2
3
4
5
6
7
8
9


ppm
20
40
60
80
100
120
140
160
180
200
H
O
OTBS
O
EtO
2.15

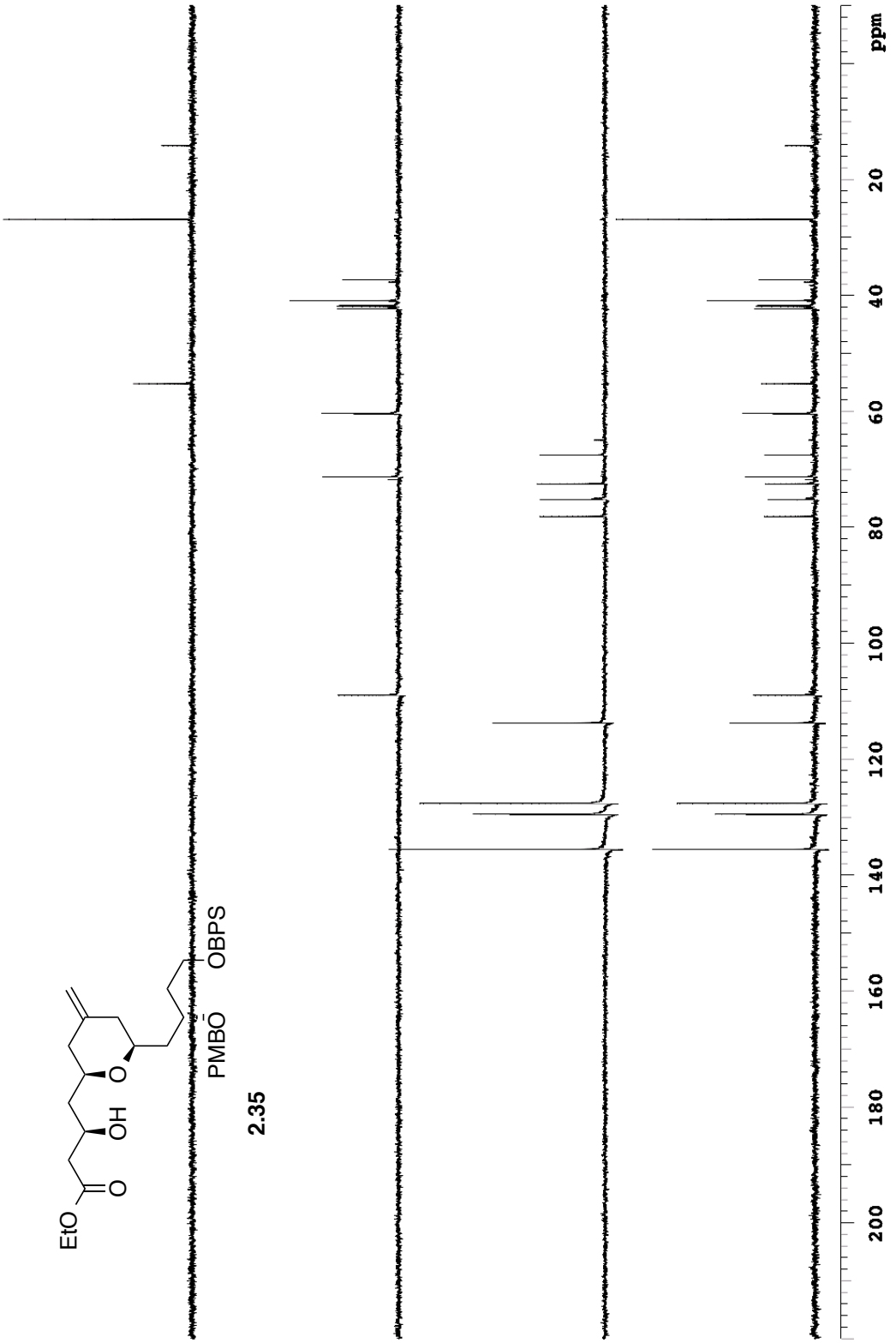

H
O
OTBS
O
EtO
2.15
200
180
160
140
120
100
80
60
40
20
ppm

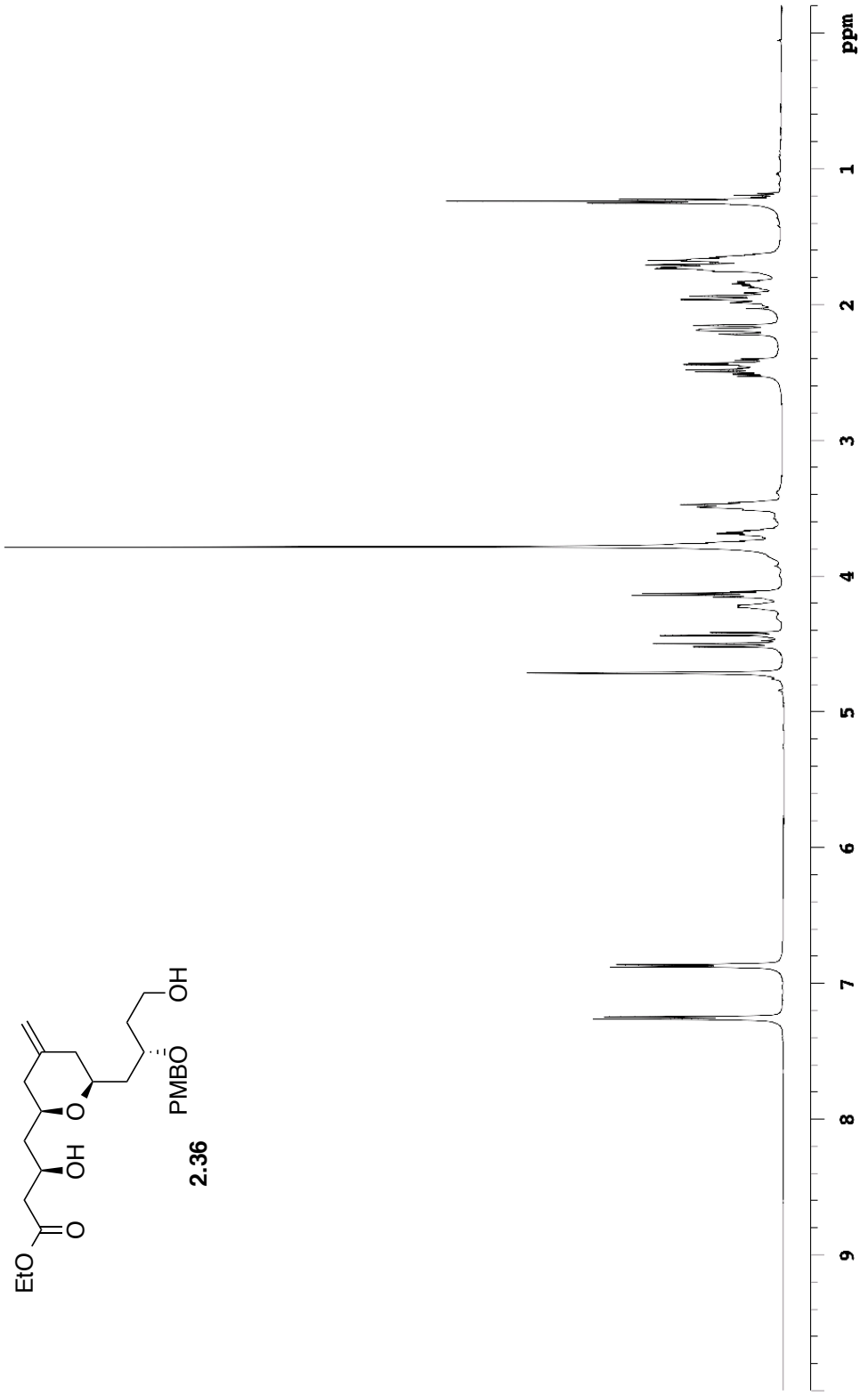


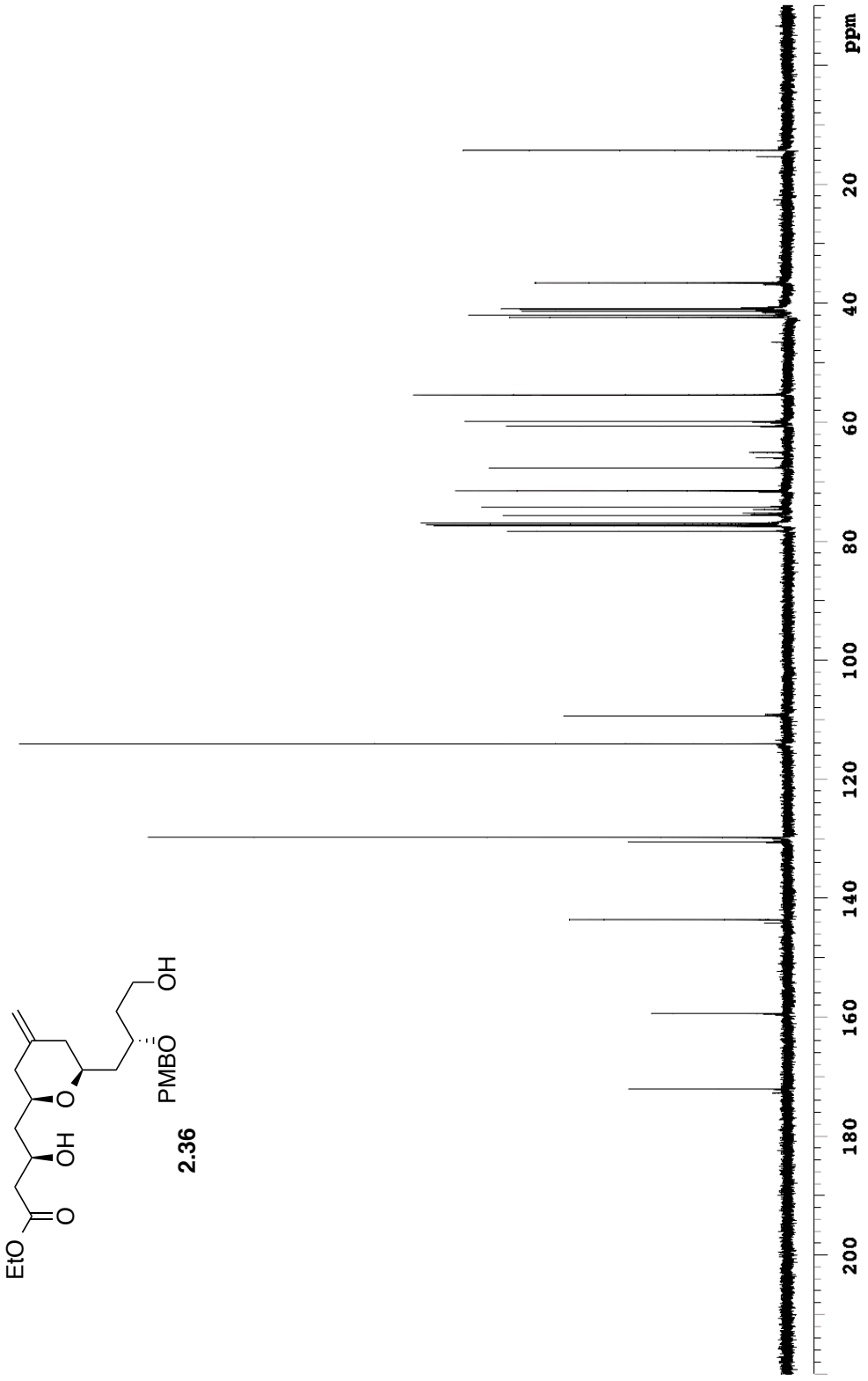
2.35



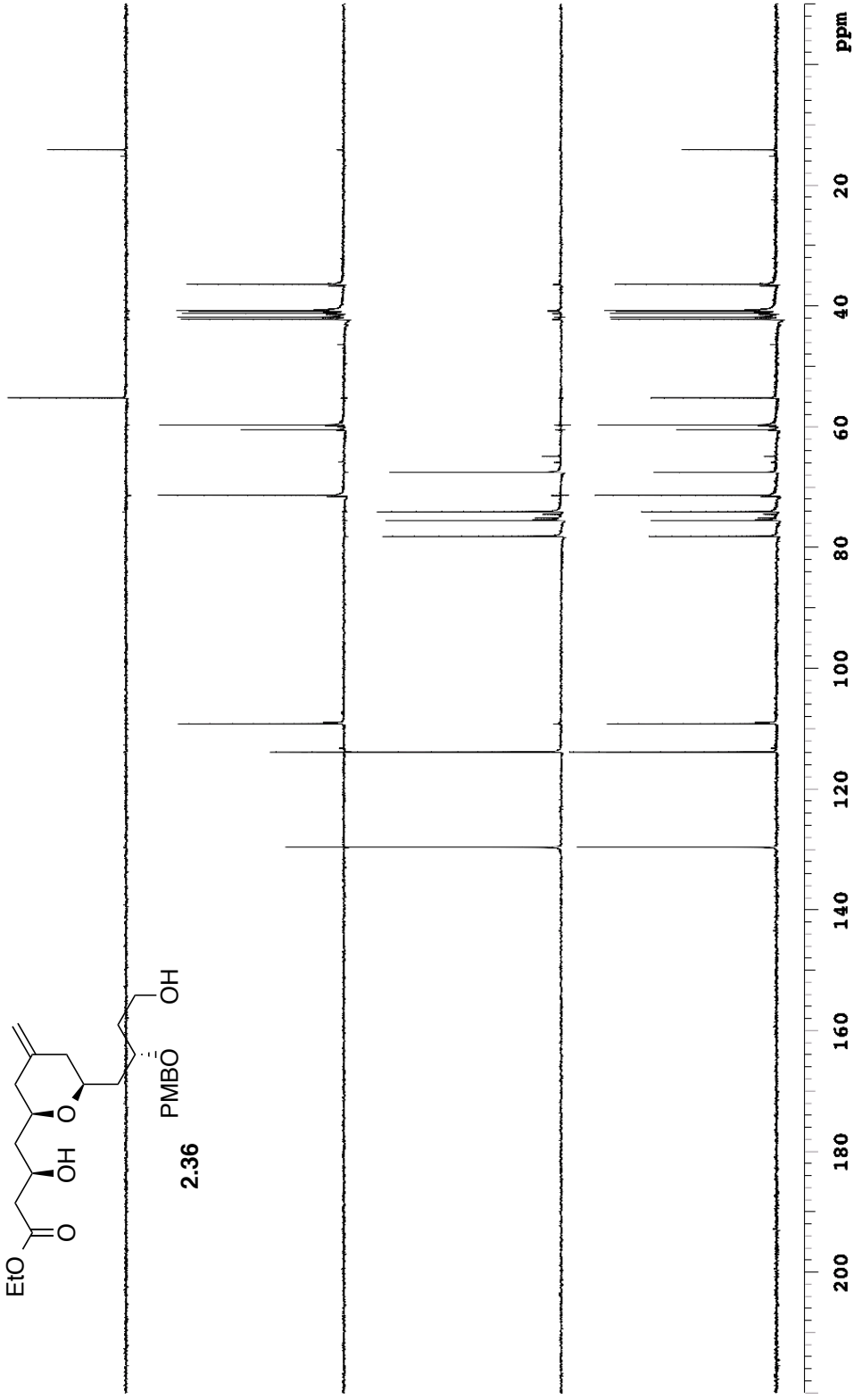
2.35

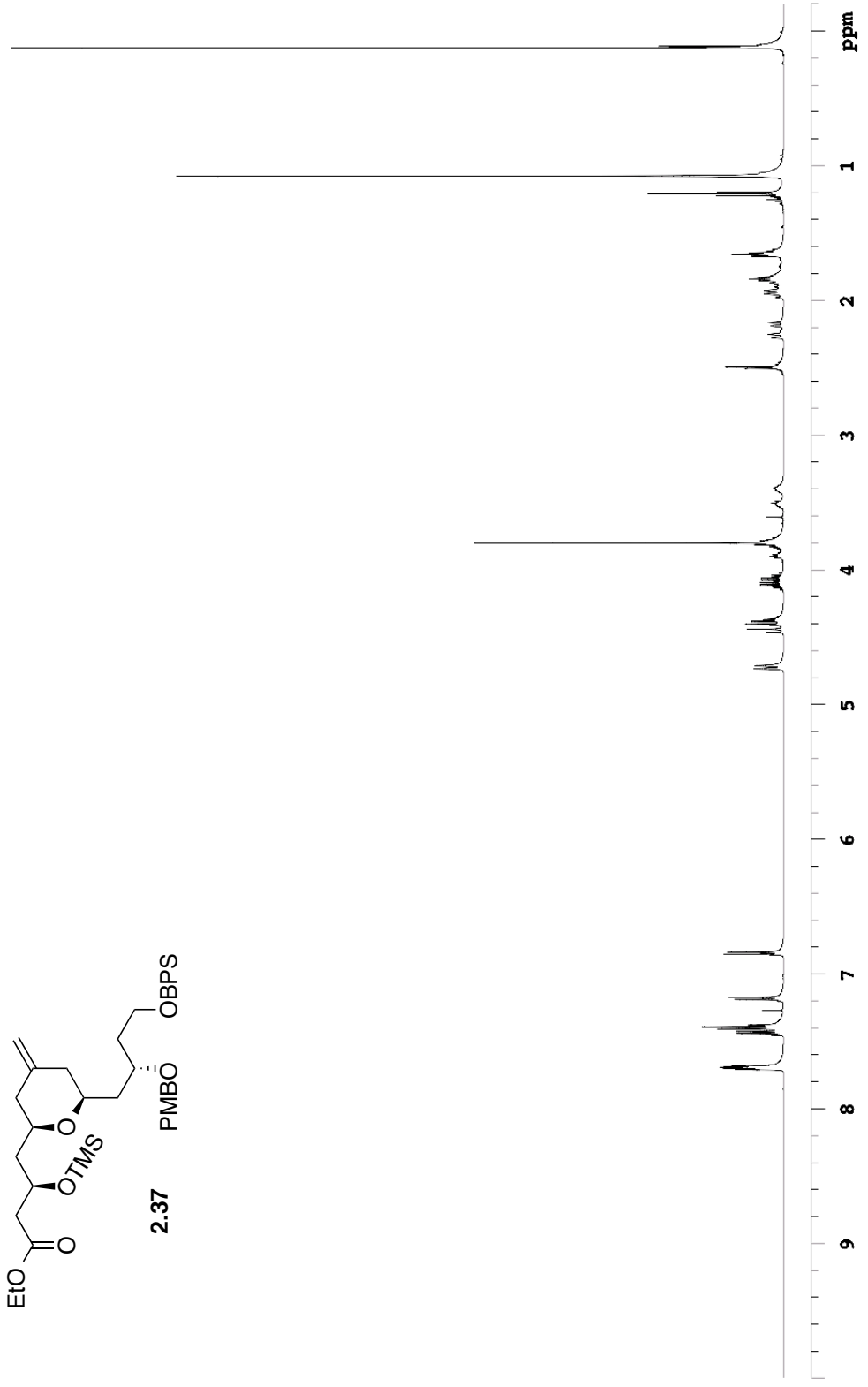

EtO
O
OH
O
PMBO
OBPS
2.35
200
180
160
140
120
100
80
60
40
20
ppm

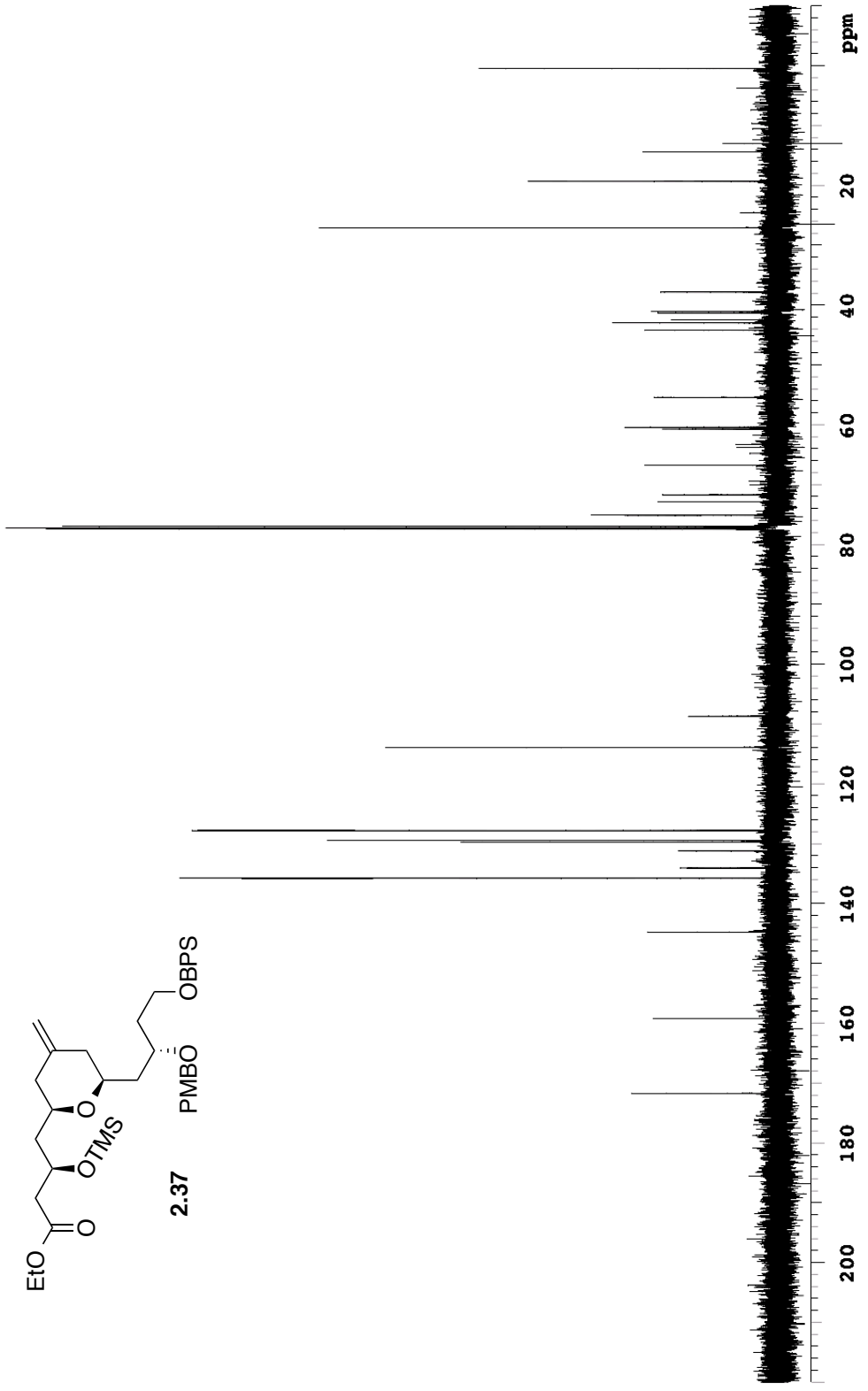

EtO
O
OH
O
PMBO
OH
2.36
ppm
1
2
3
4
5
6
7
8
9



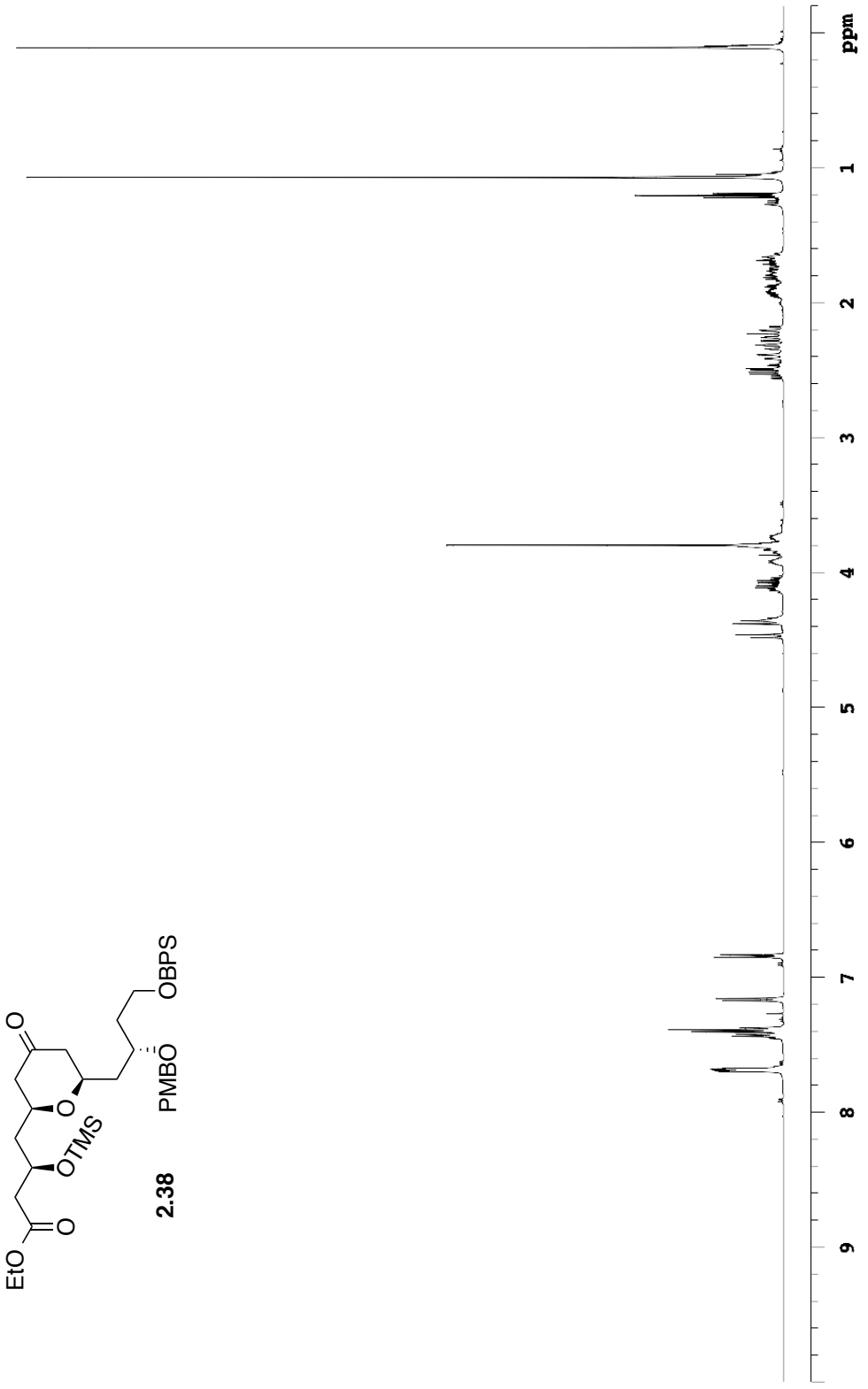
2.36

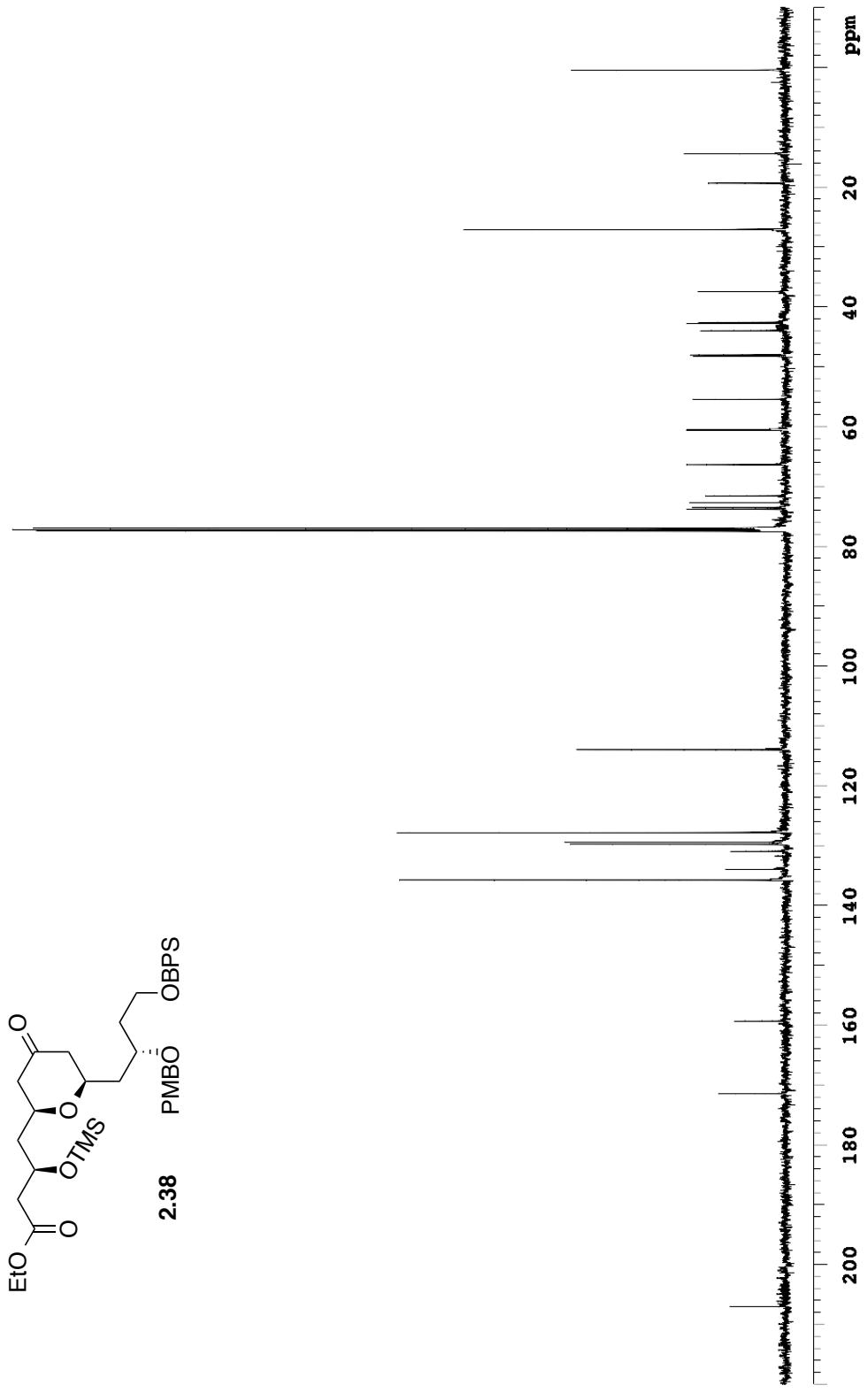

EtO
O
OH
O
PMBO
OH
2.36
ppm
200
180
160
140
120
100
80
60
40
20

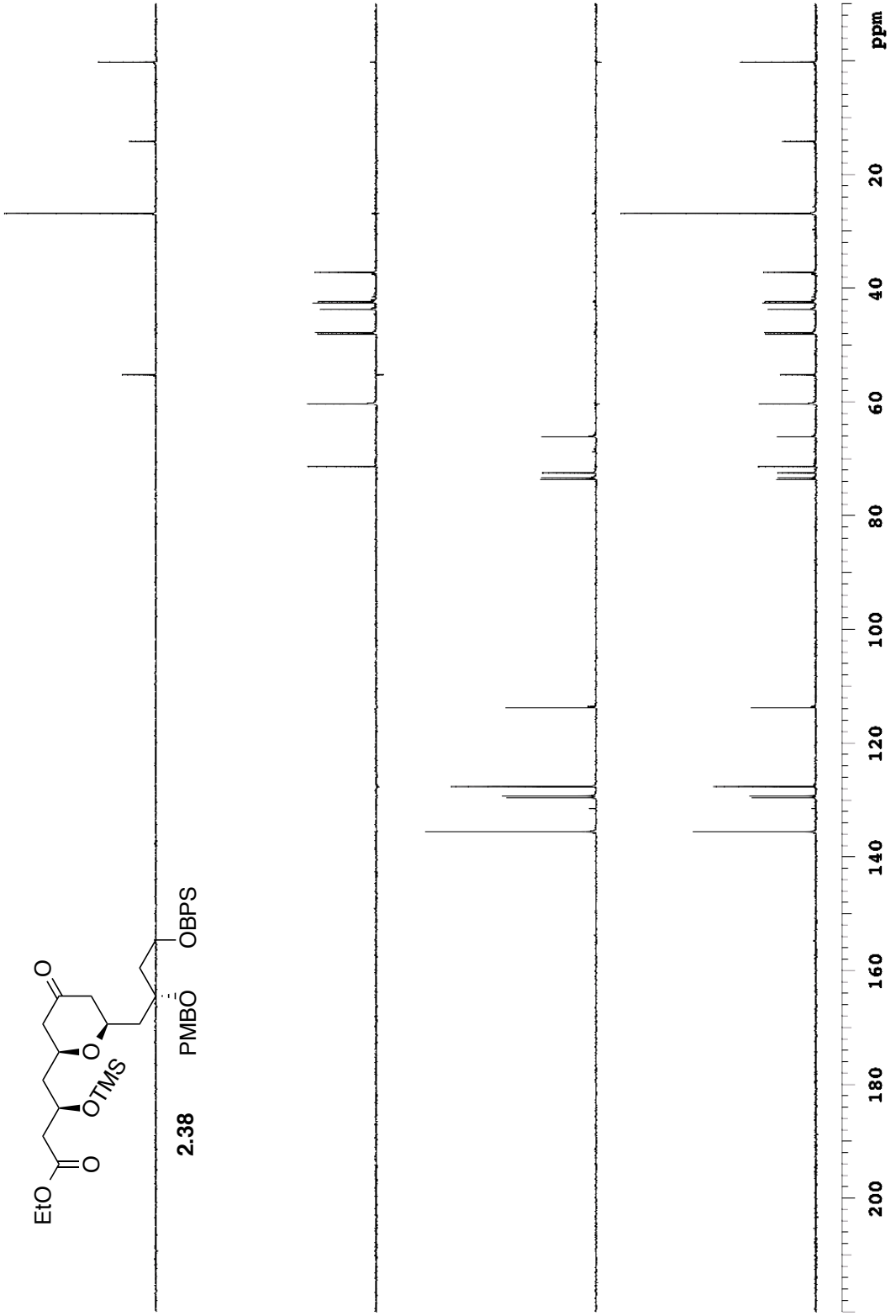

EtO
O
OTMS
O
PMBO
OBPS
2.37
ppm
1
2
3
4
5
6
7
8
9

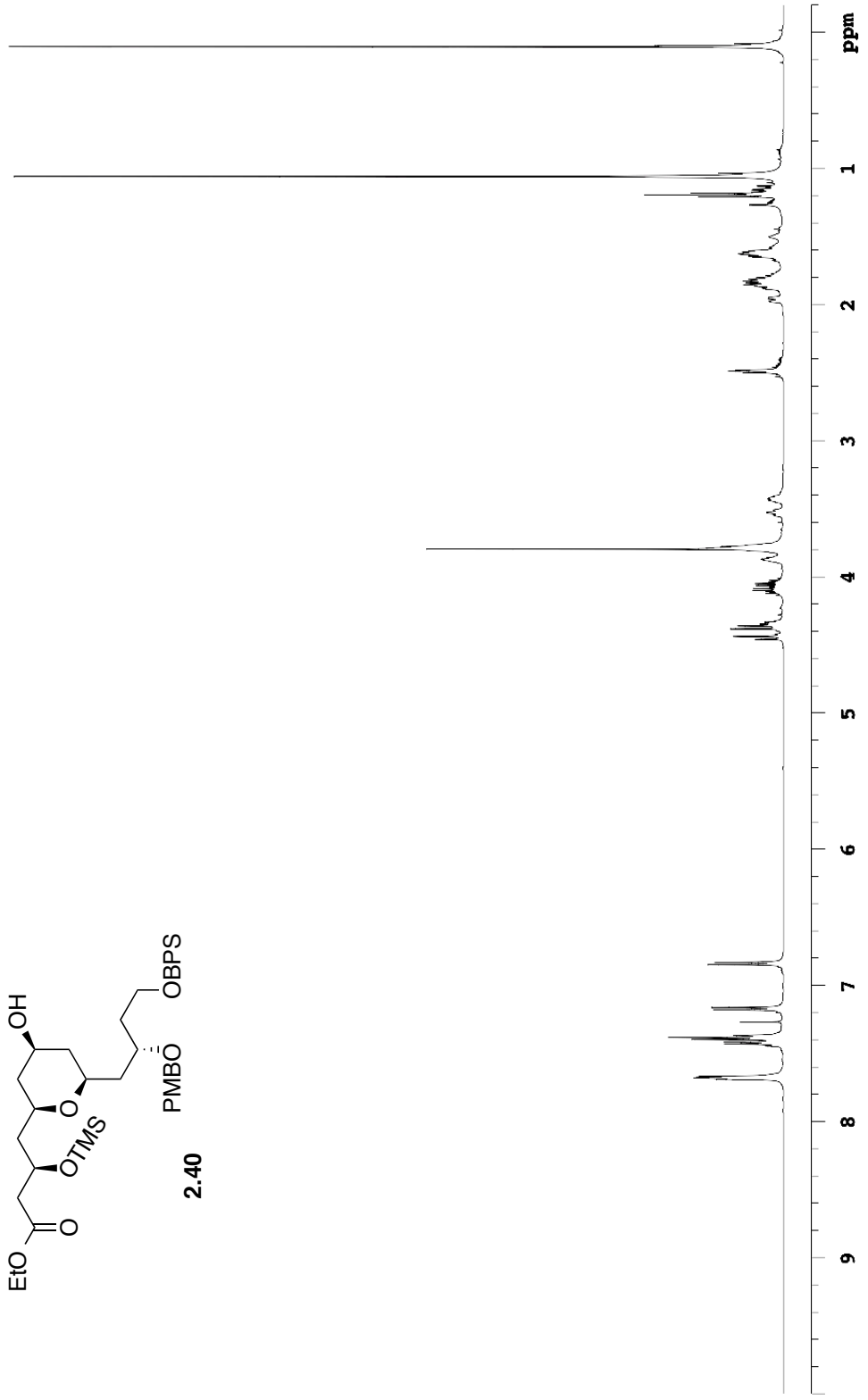


2.37

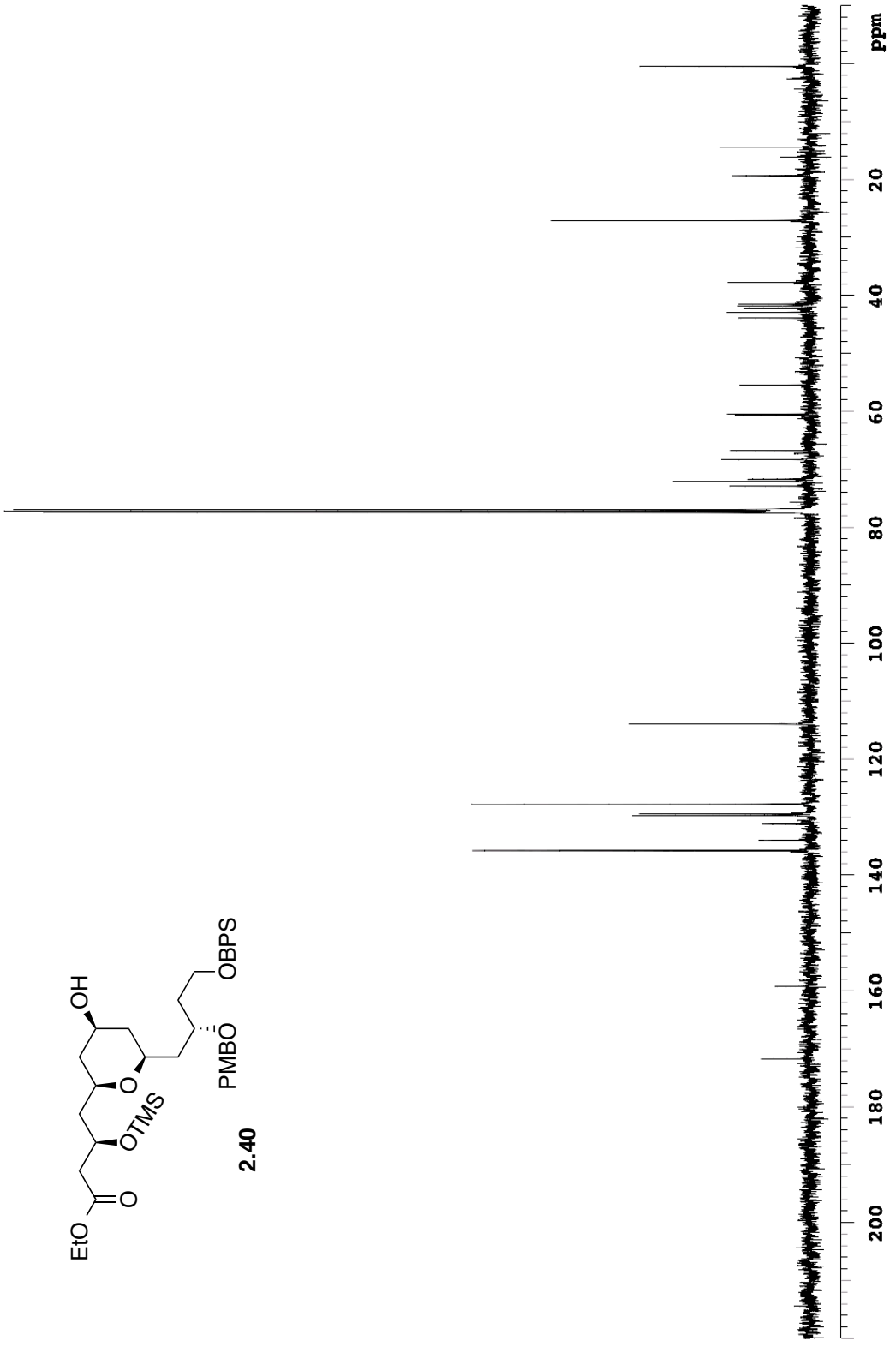

EtO
O
O
OTMS
O
PMBO
OBPS
2.38
ppm
1
2
3
4
5
6
7
8
9


EtO
O
O
OTMS
O
PMBO
OBPS
O
2.38
ppm
200
180
160
140
120
100
80
60
40
20

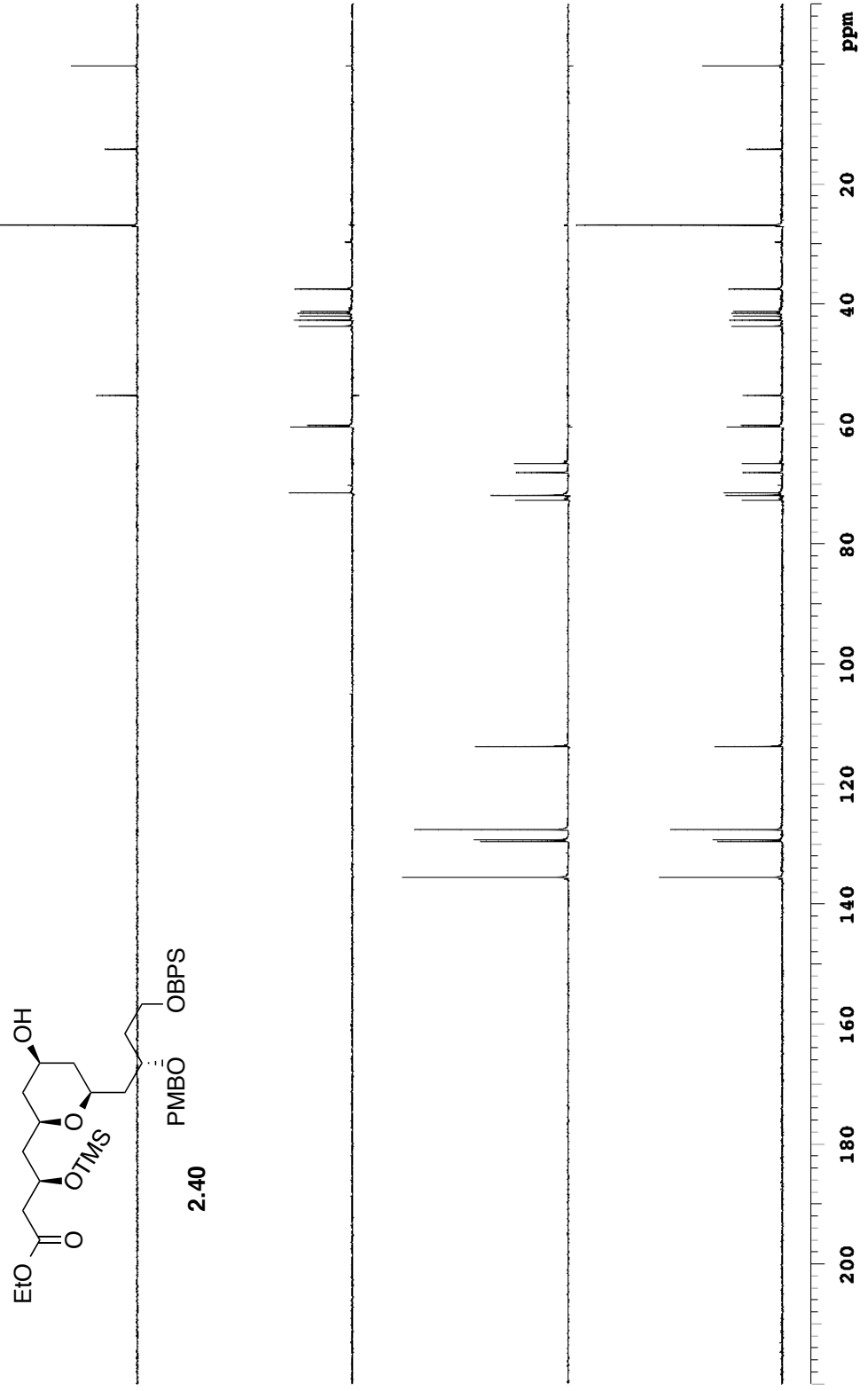

EtO
O
O
OTMS
O
PMBO
OBPS
2.38
ppm
200
180
160
140
120
100
80
60
40
20

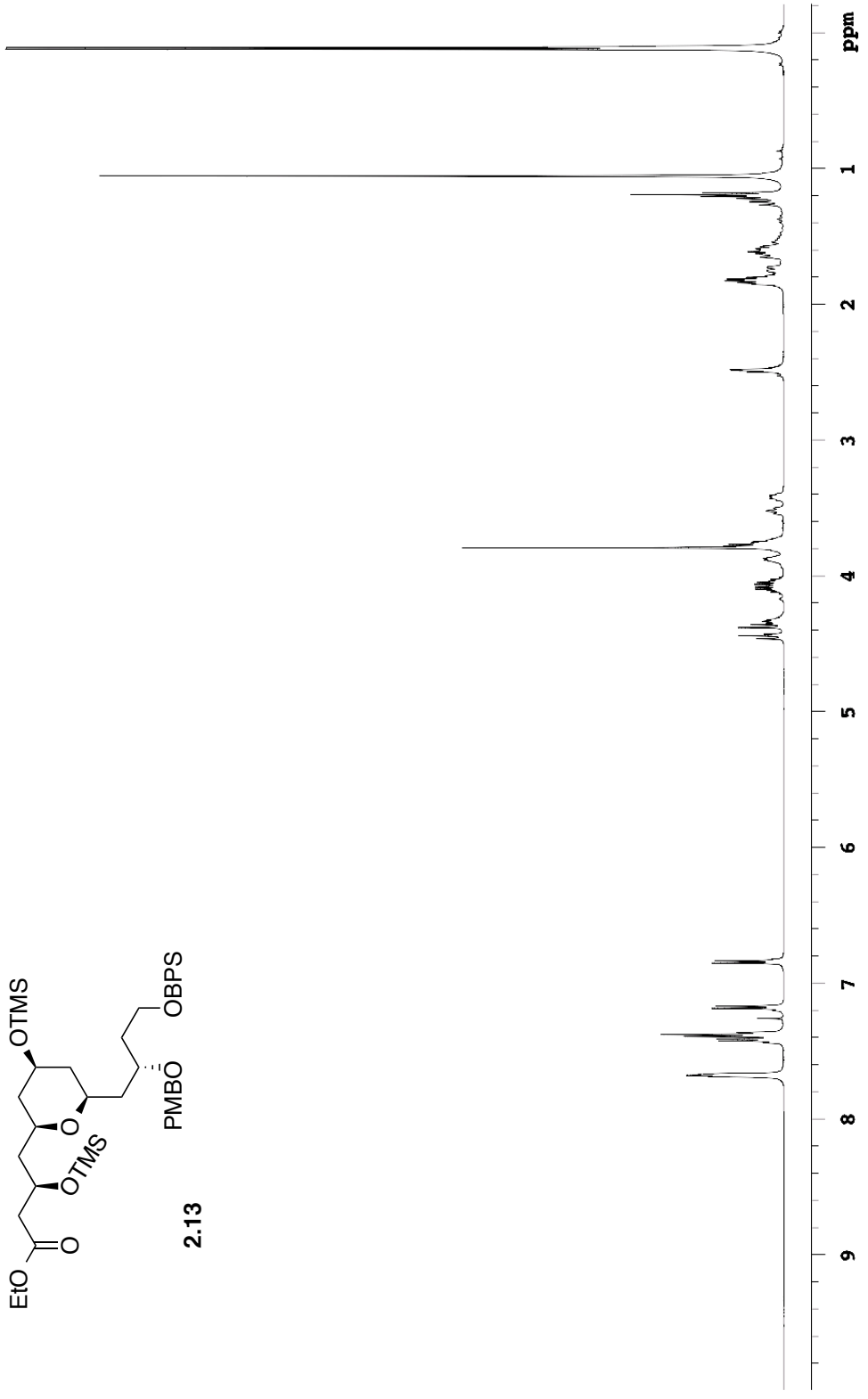


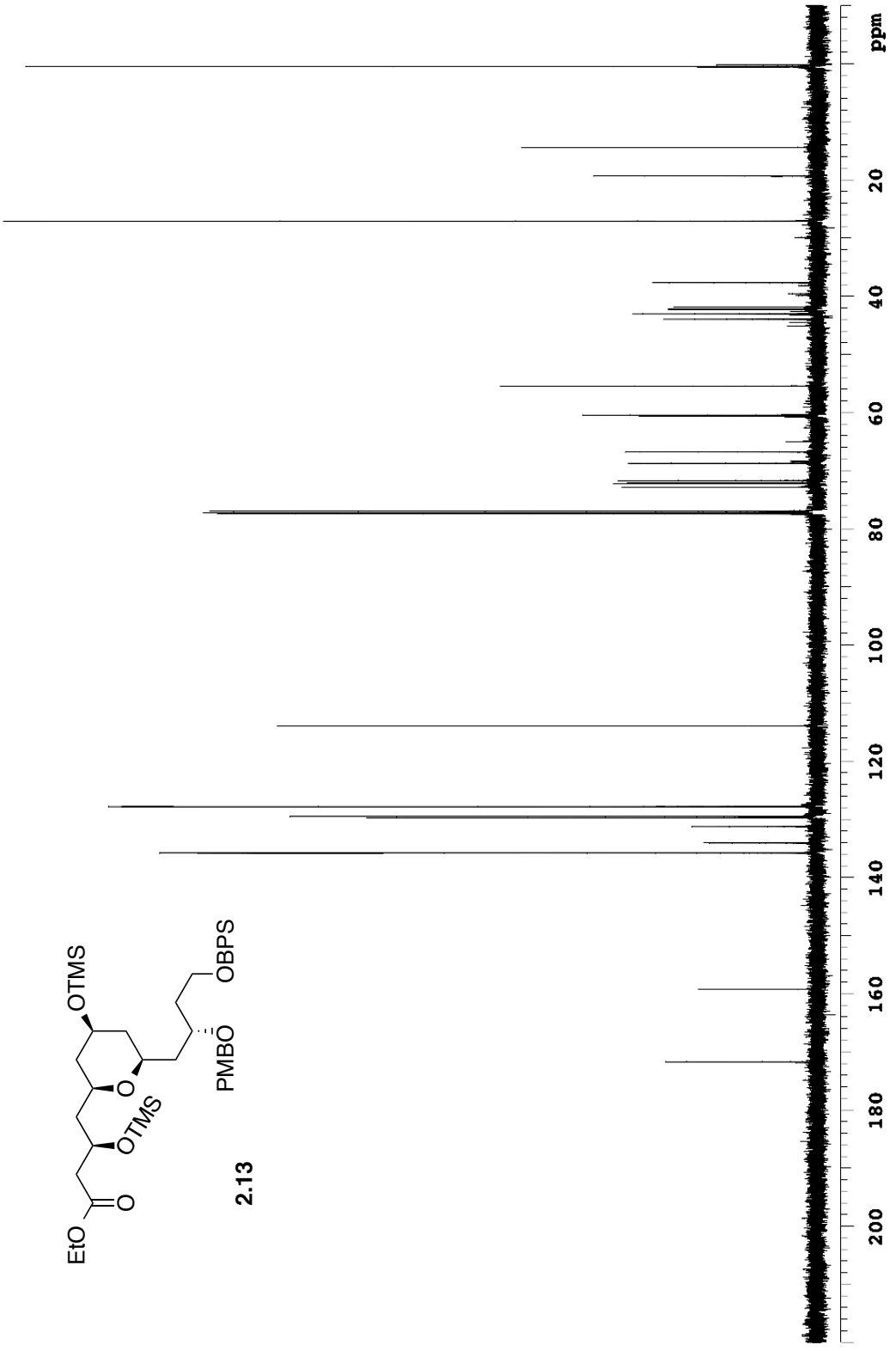
2.40

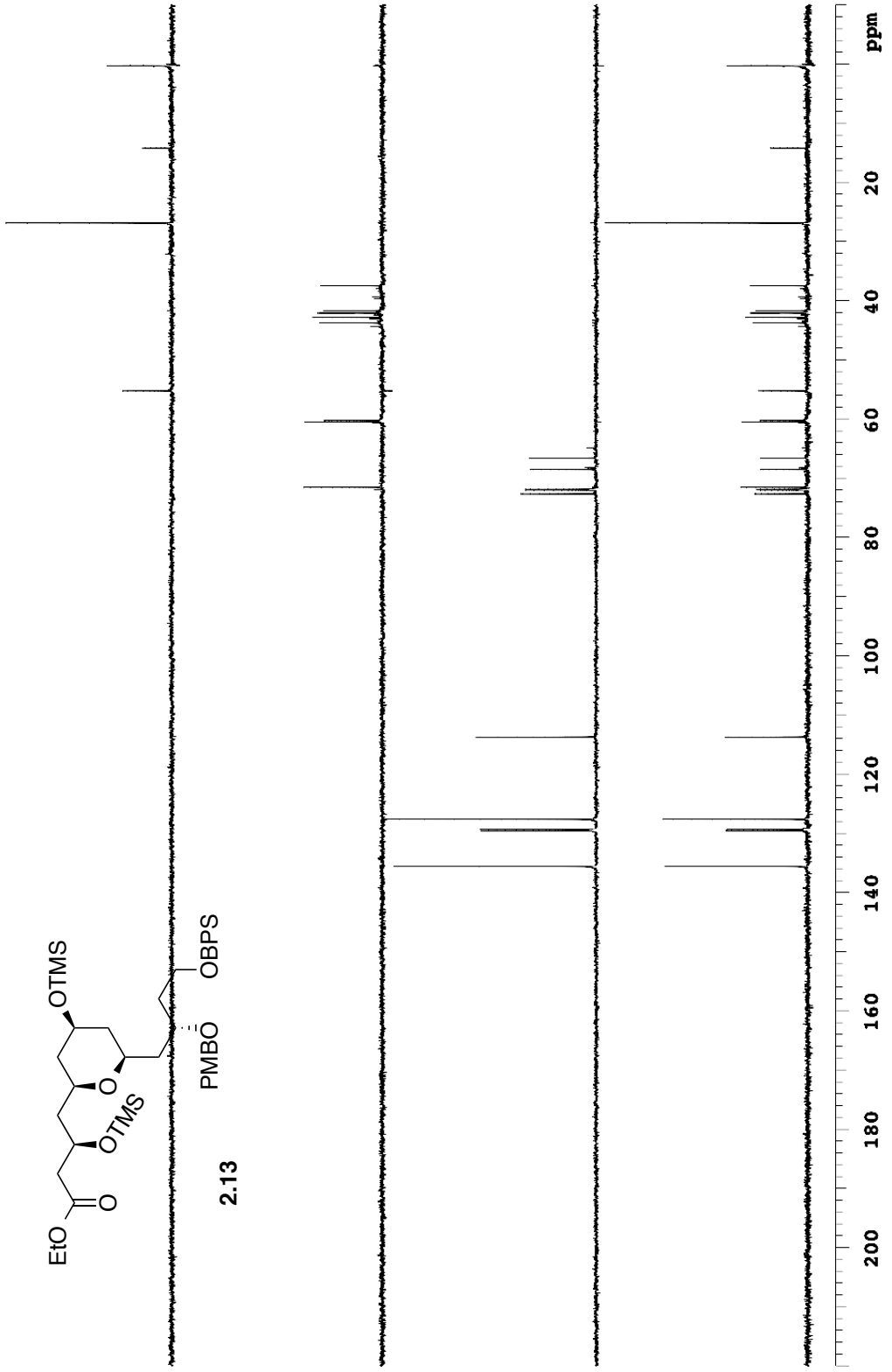


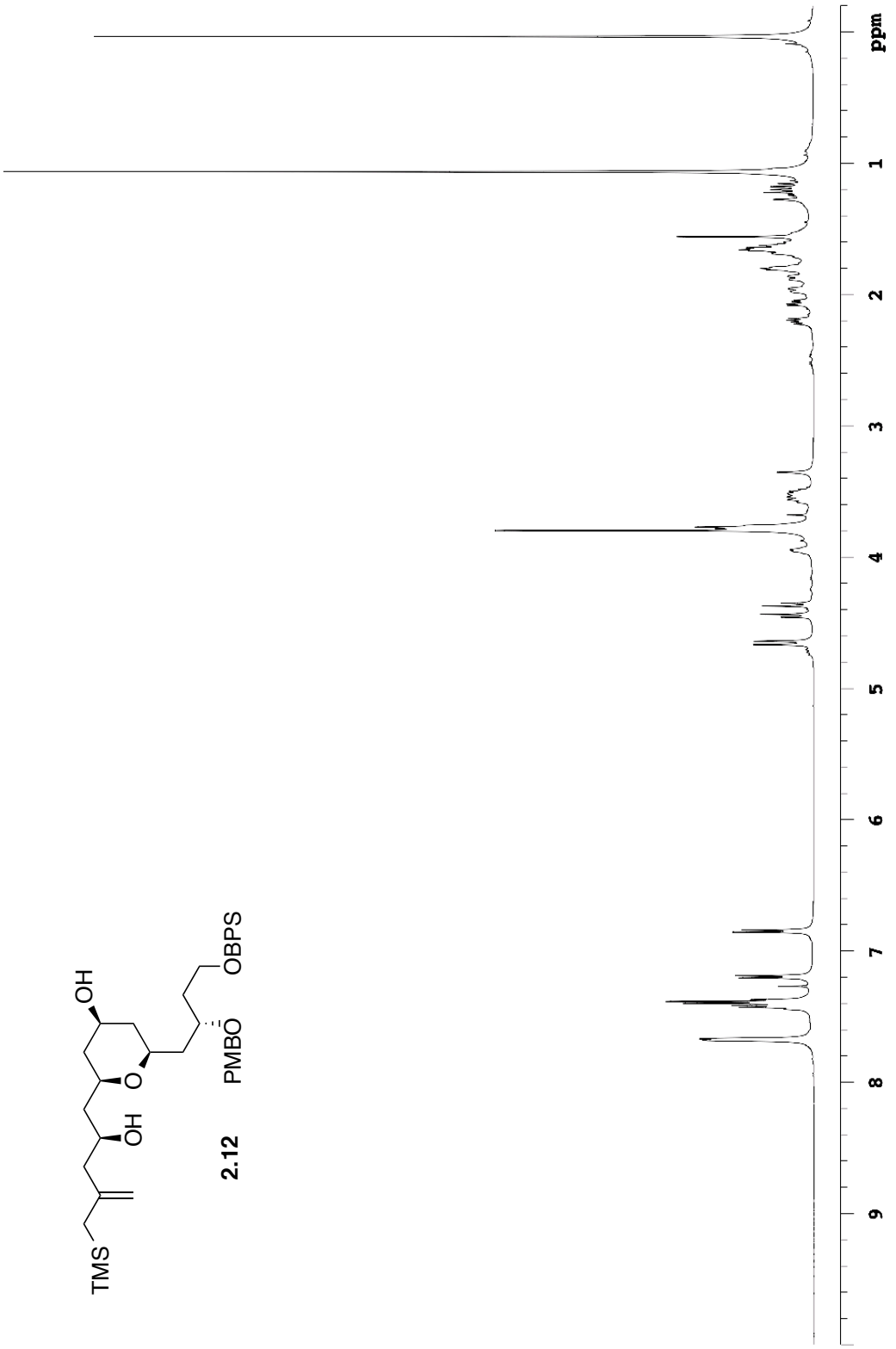
2.40

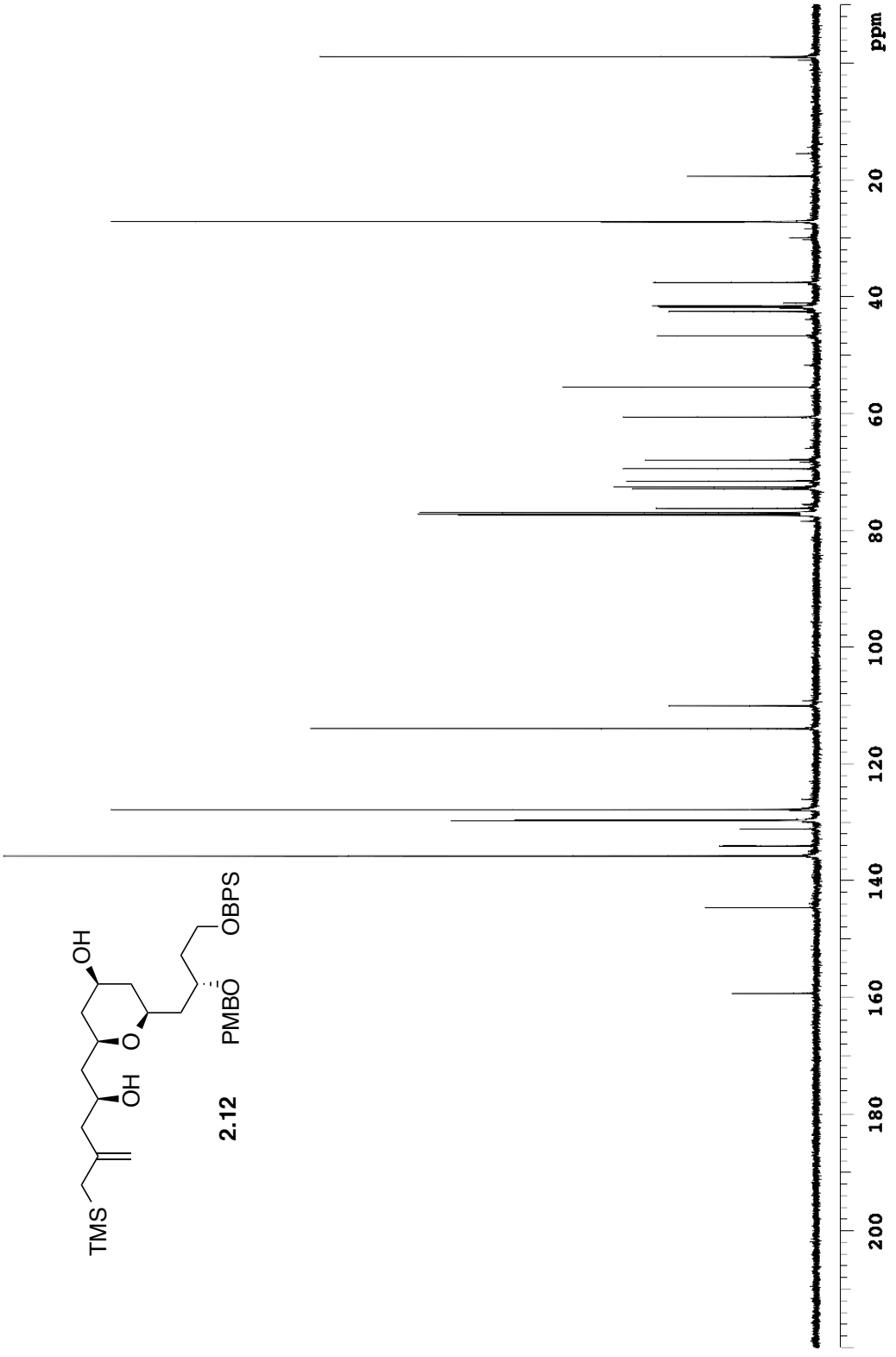

OH
O
OTMS
PMBO
OBPS
EtO
O
2.40
ppm
200
180
160
140
120
100
80
60
40
20

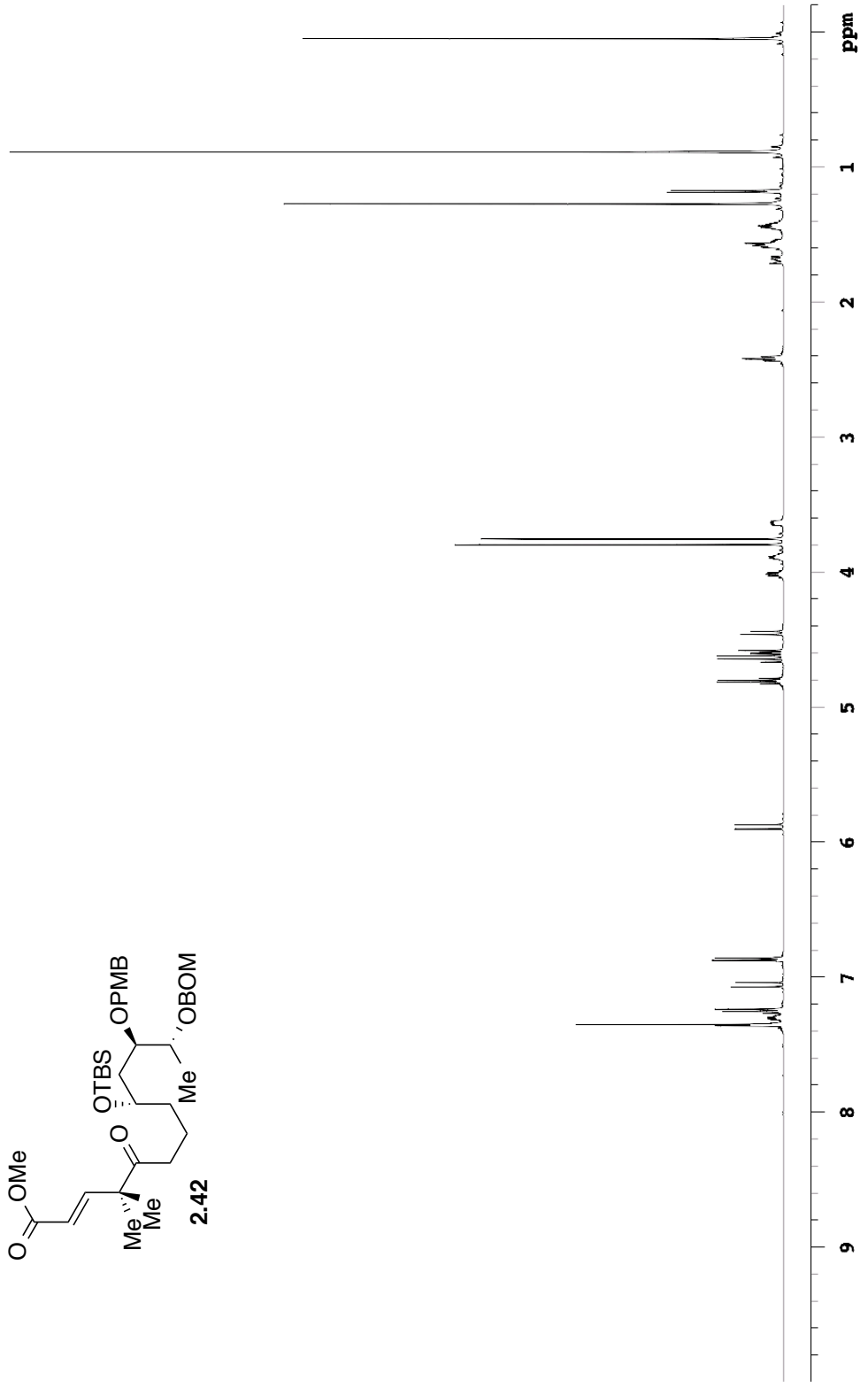

OTMS
OTMS
O
O
EtO
PMBO
OBPS
2.13
ppm
1
2
3
4
5
6
7
8
9

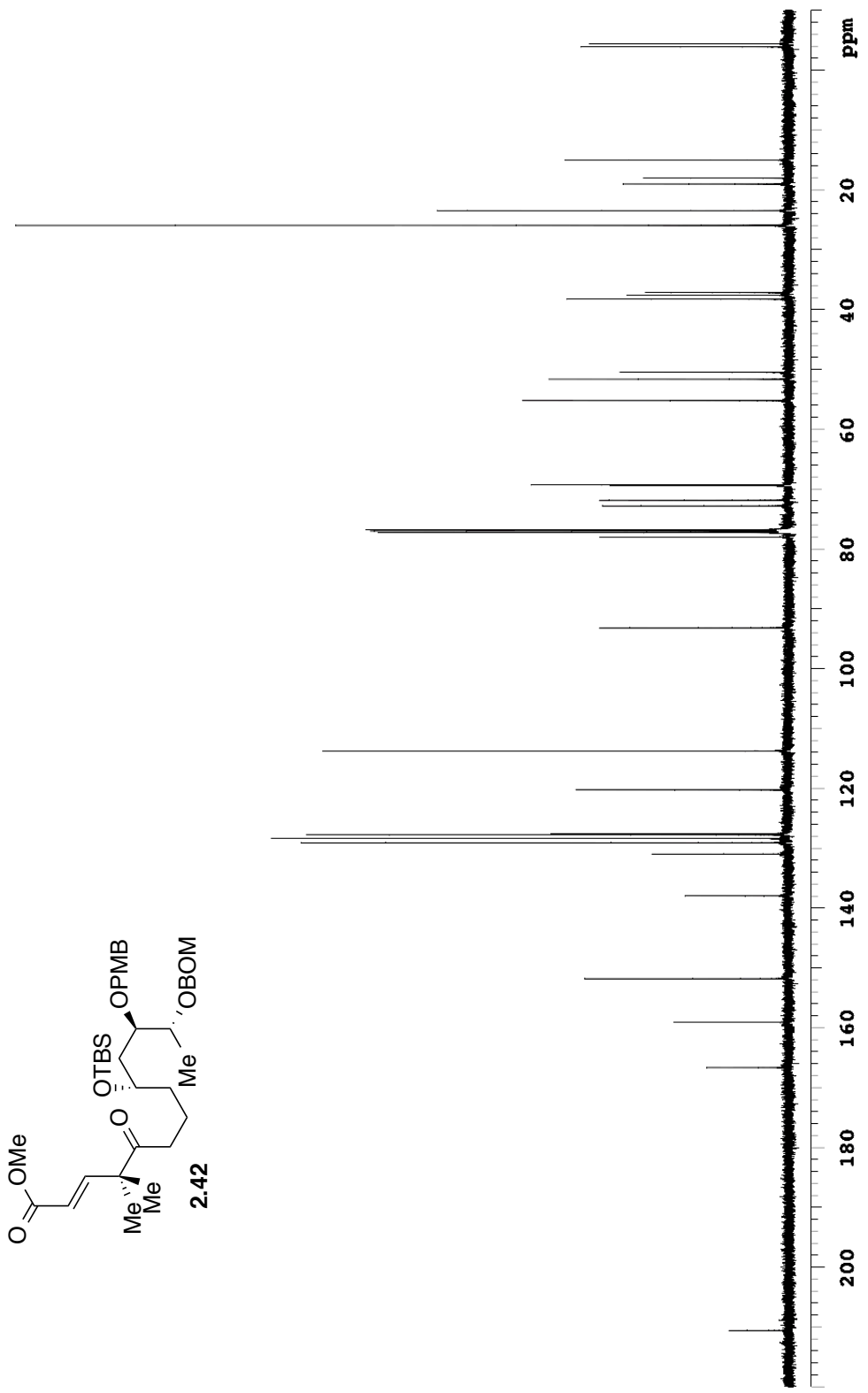

OTMS
OBPS
PMBO
OTMS
O
EtO
O
2.13
ppm
200
180
160
140
120
100
80
60
40
20

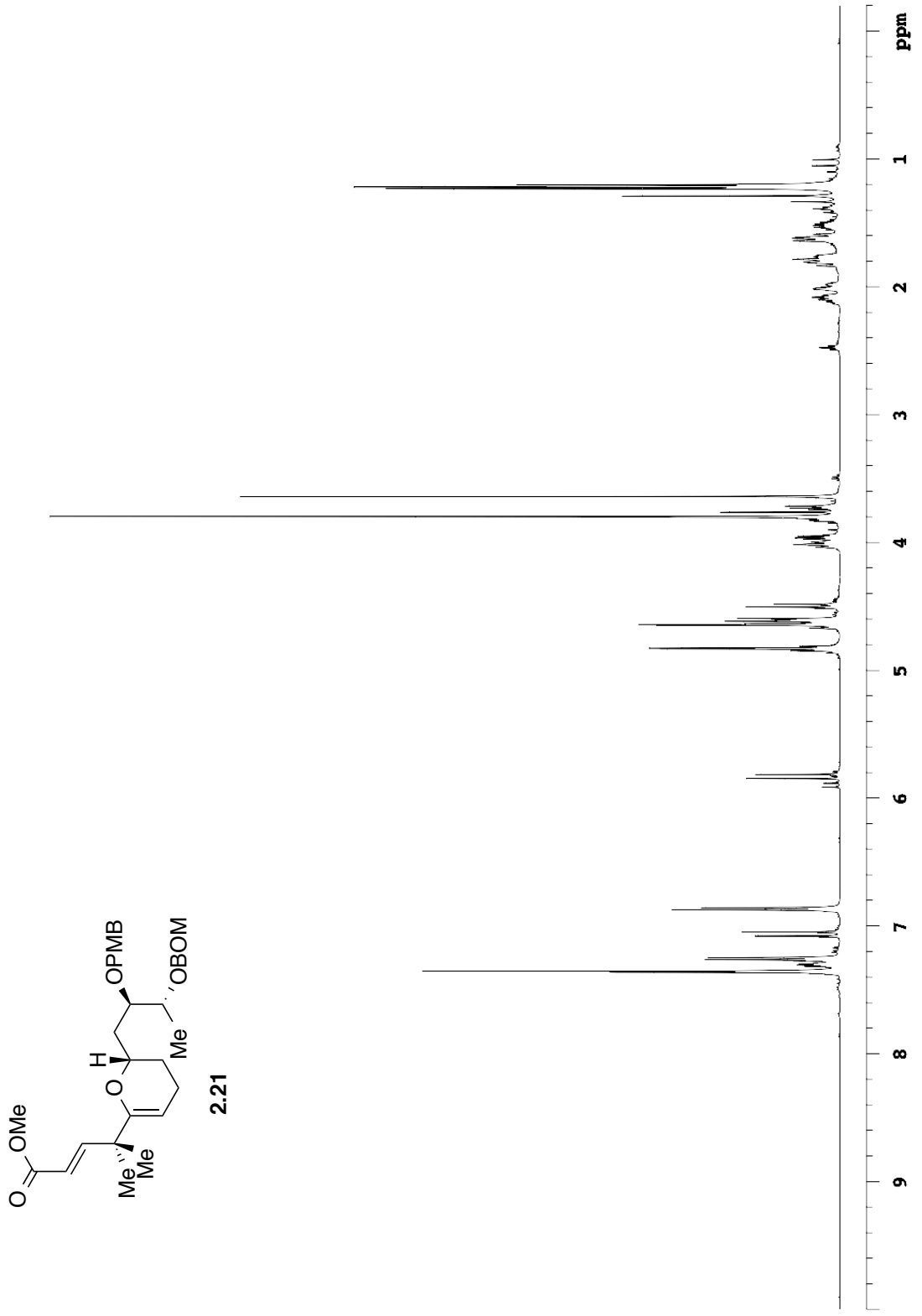

OTMS
OTMS
O
O
EtO
PMBO
OBPS
2.13
200
180
160
140
120
100
80
60
40
20
ppm

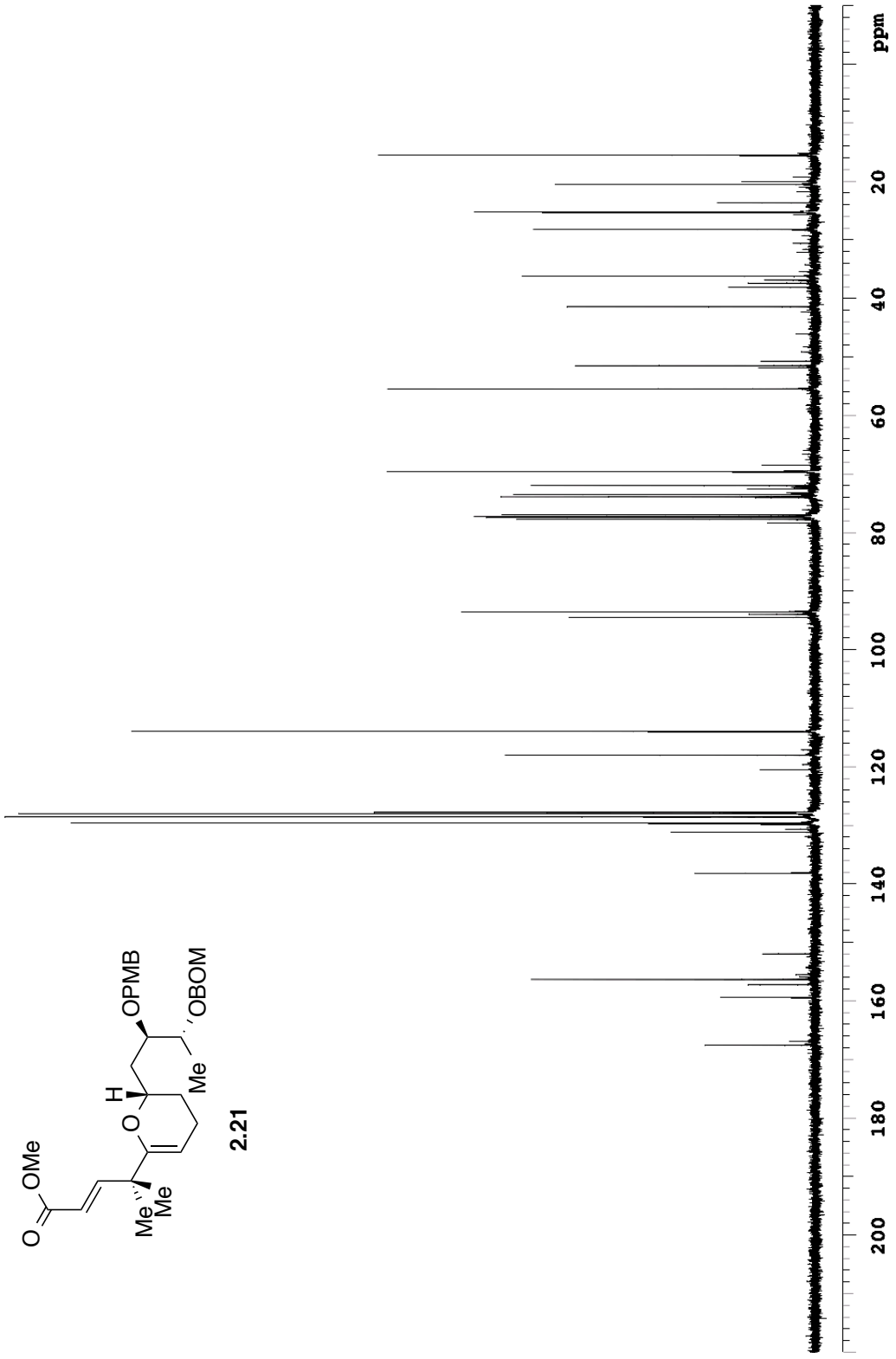

TMS
OH
OH
O
PMBO
OBPS
2.12
ppm
1
2
3
4
5
6
7
8
9

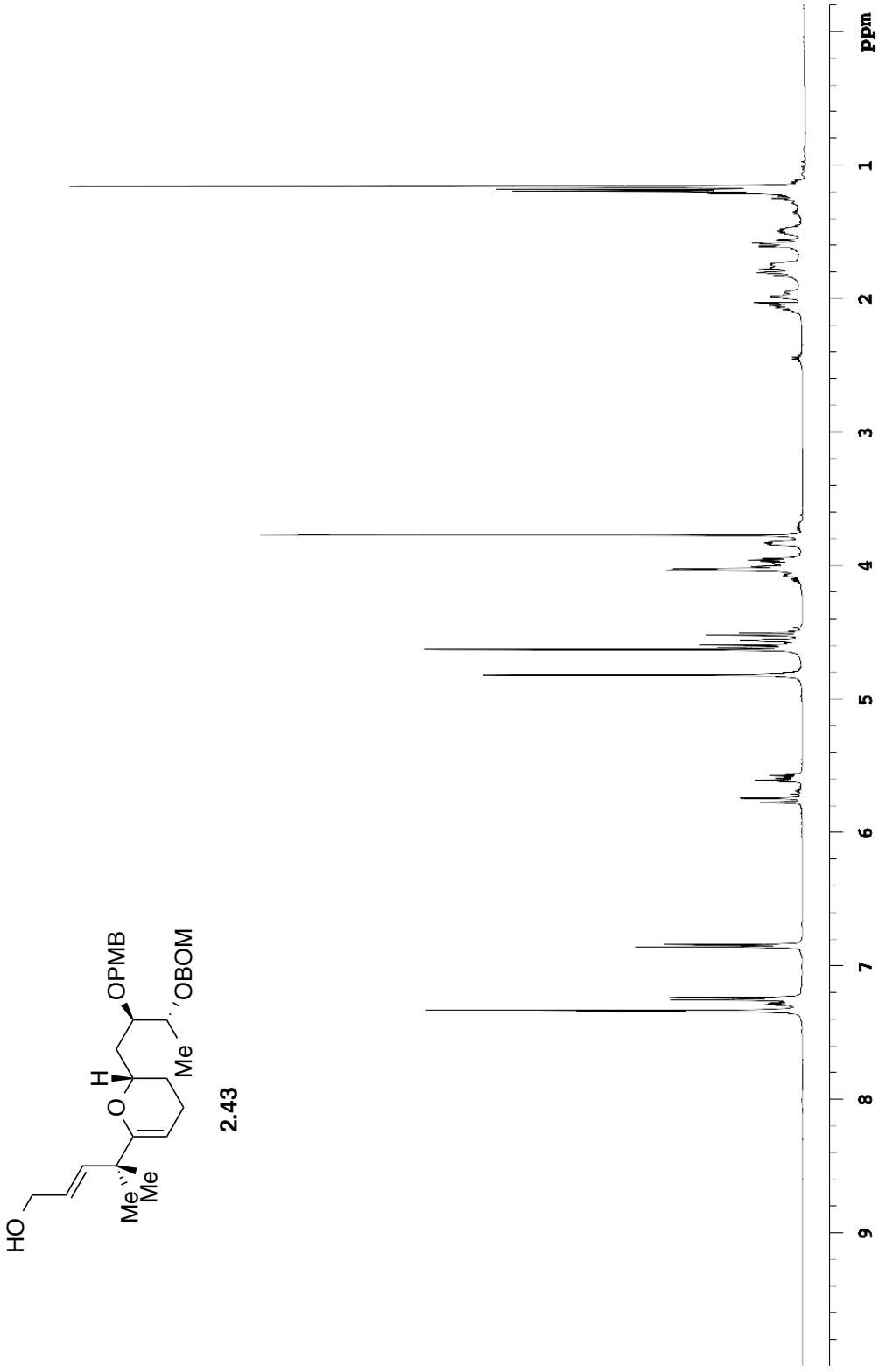

TMS
OH
OH
O
PMBO
OBPS
2.12
ppm
20
40
60
80
100
120
140
160
180
200

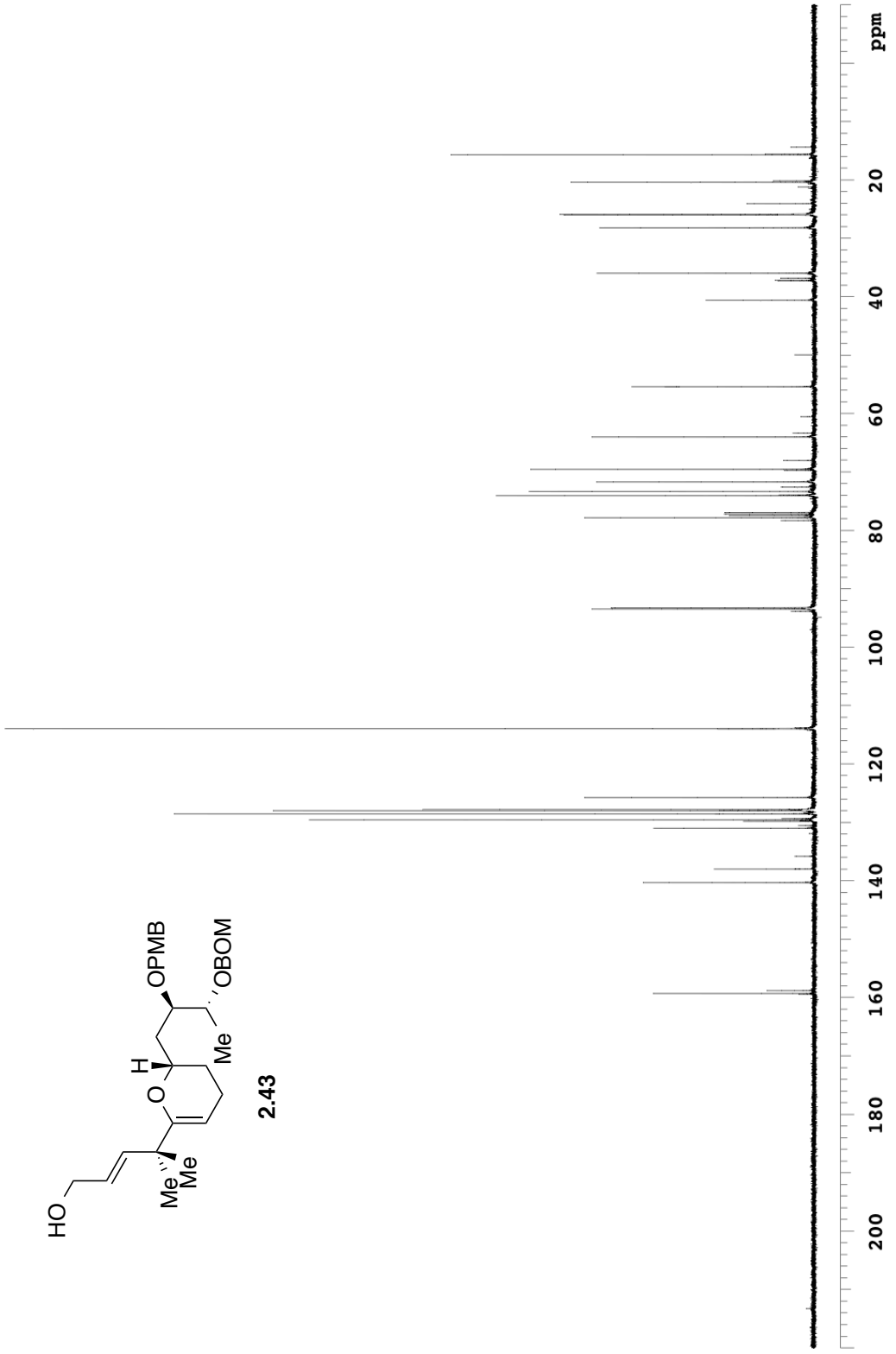

OMe
O
Me
Me
O
OTBS
OPMB
Me
OBOM
2.42
ppm
1
2
3
4
5
6
7
8
9


OMe
O
Me
Me
O
OTBS
OPMB
Me
OBOM
2.42
ppm
20
40
60
80
100
120
140
160
180
200

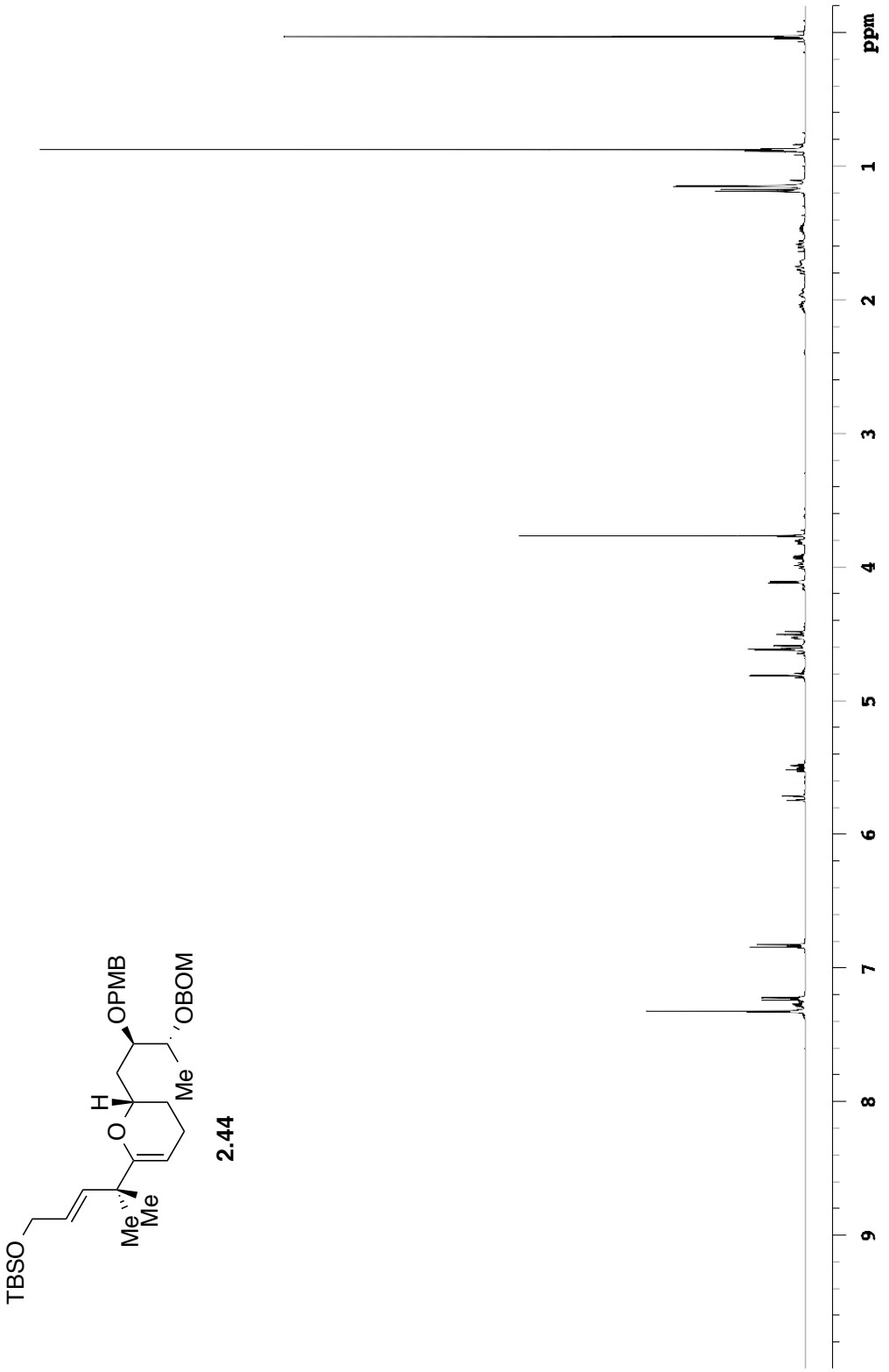

OMe
O
Me
Me
O
H
OPMB
Me
OBOM
2.21
ppm
1
2
3
4
5
6
7
8
9


OMe
O
Me
Me
H
O
OPMB
OBOM
Me
2.21
ppm
200
180
160
140
120
100
80
60
40
20

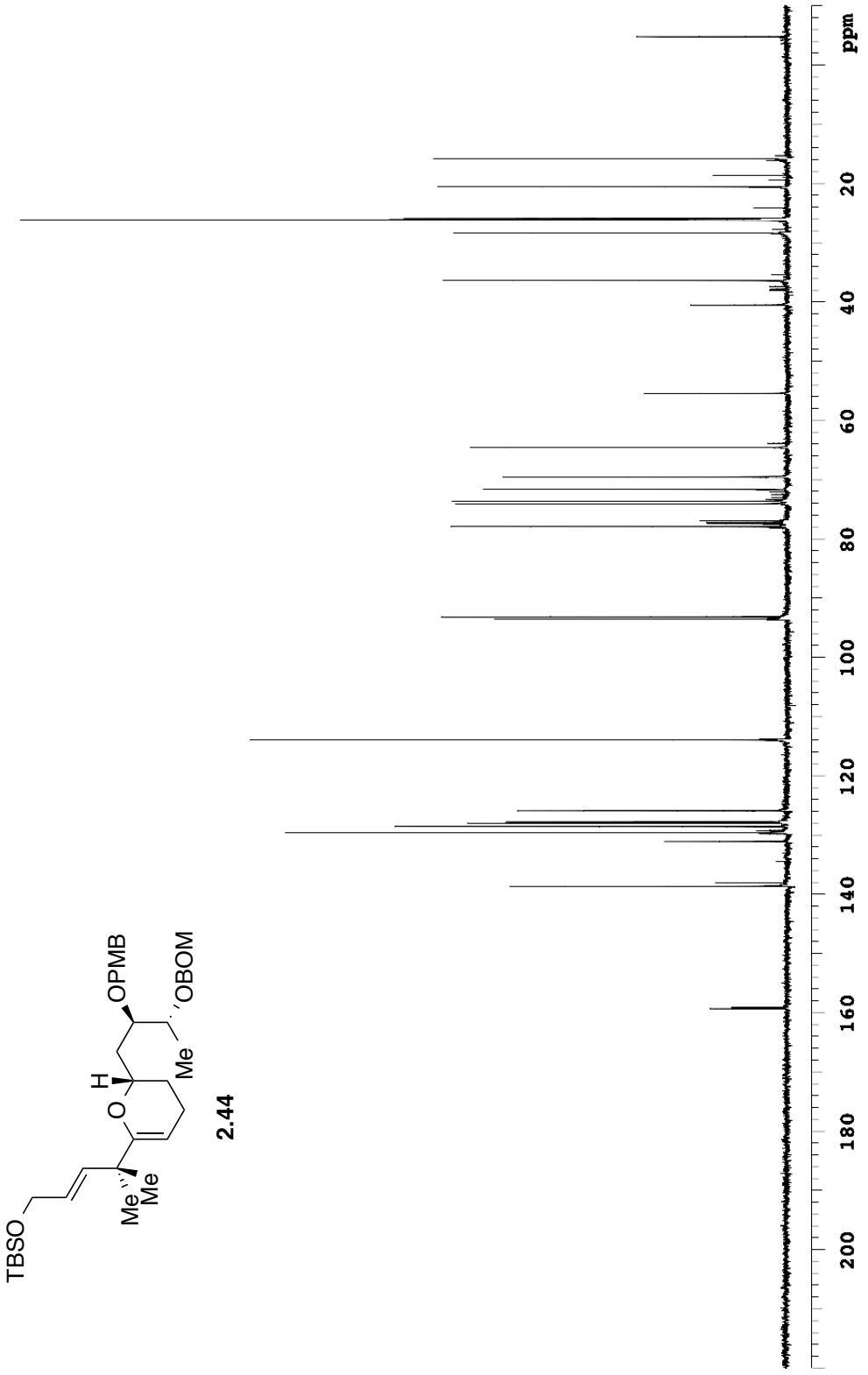

HO
Me
Me
O
H
OPMB
OBOM
Me
2.43
ppm
1
2
3
4
5
6
7
8
9

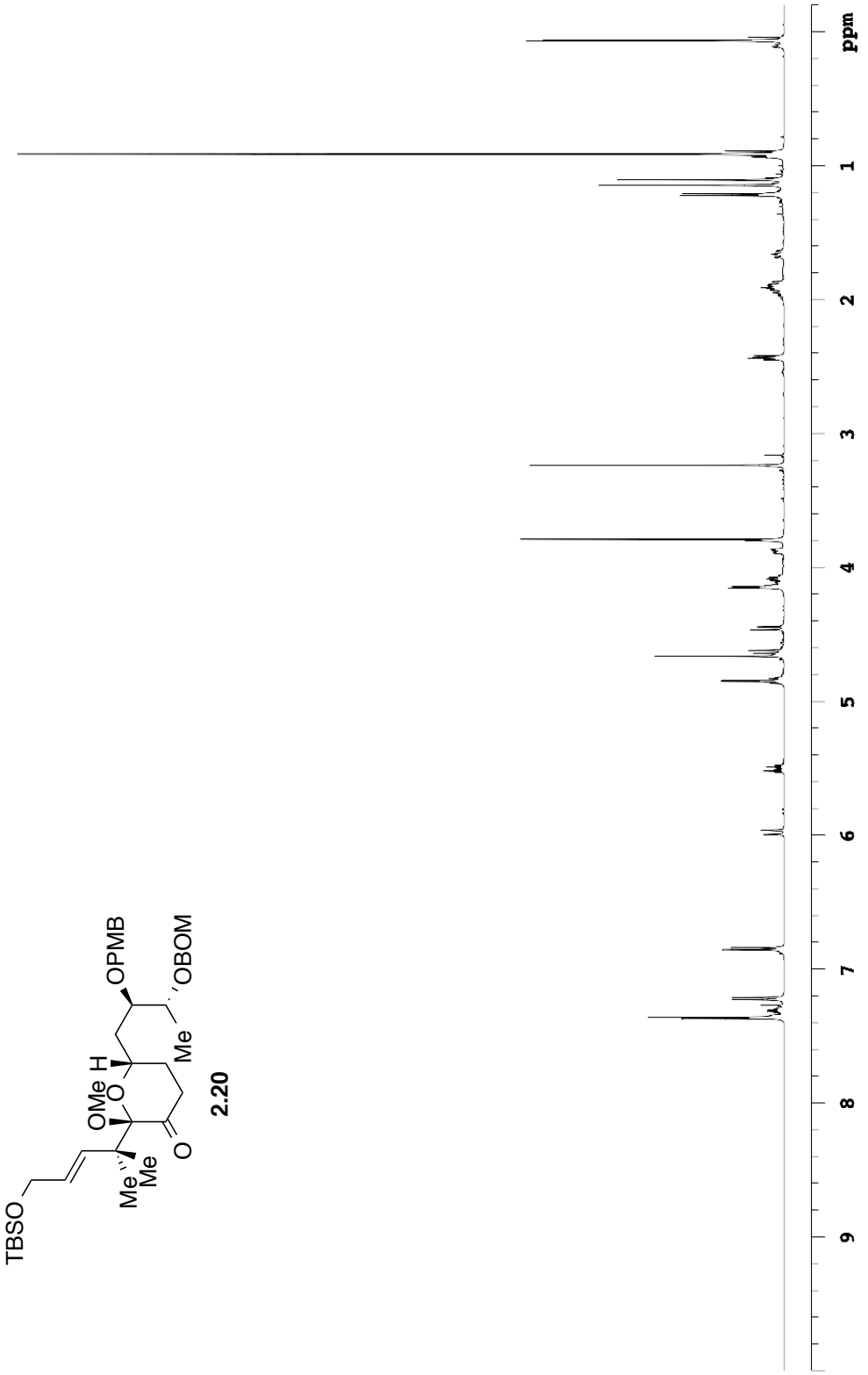


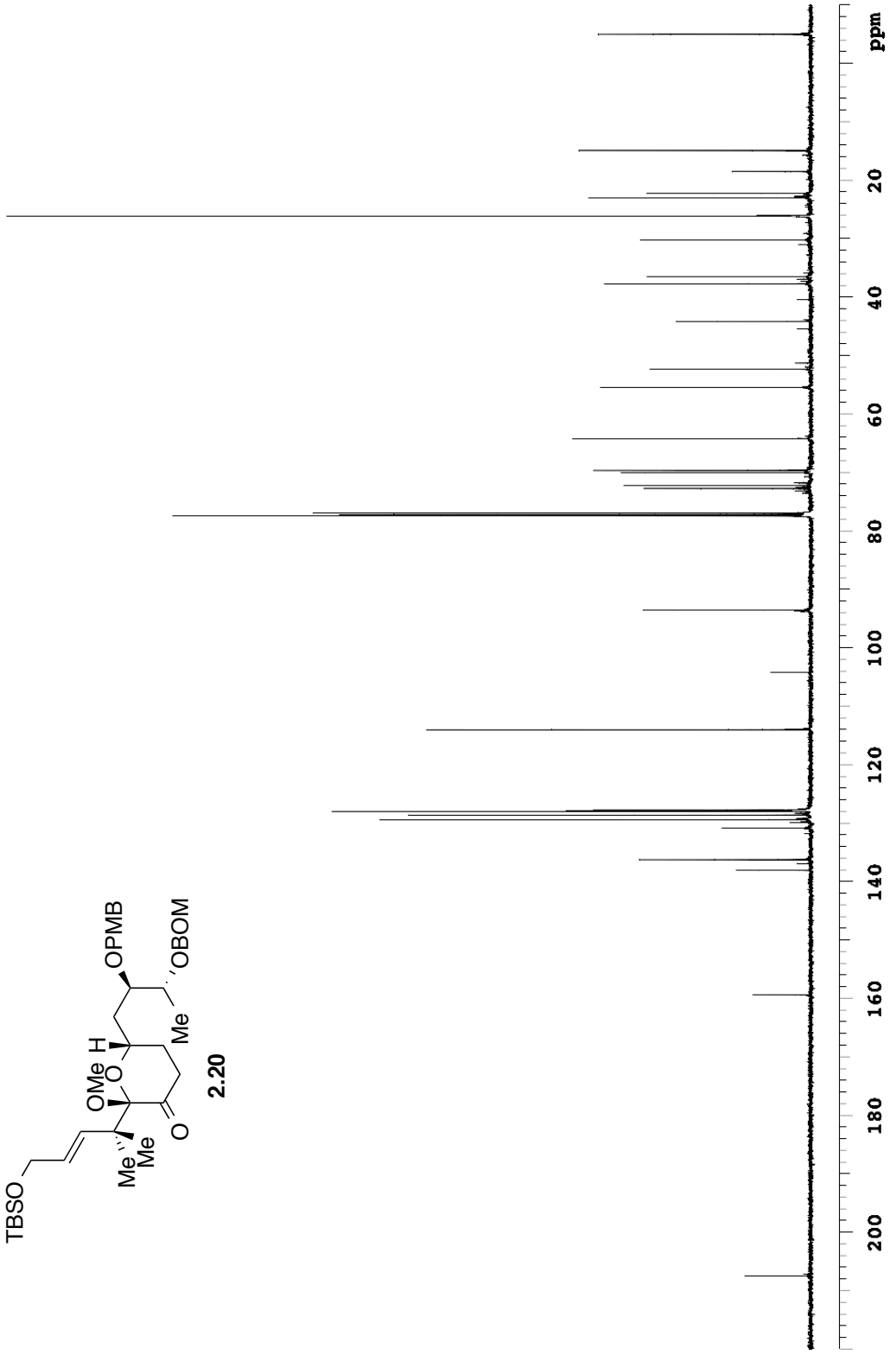
2.43

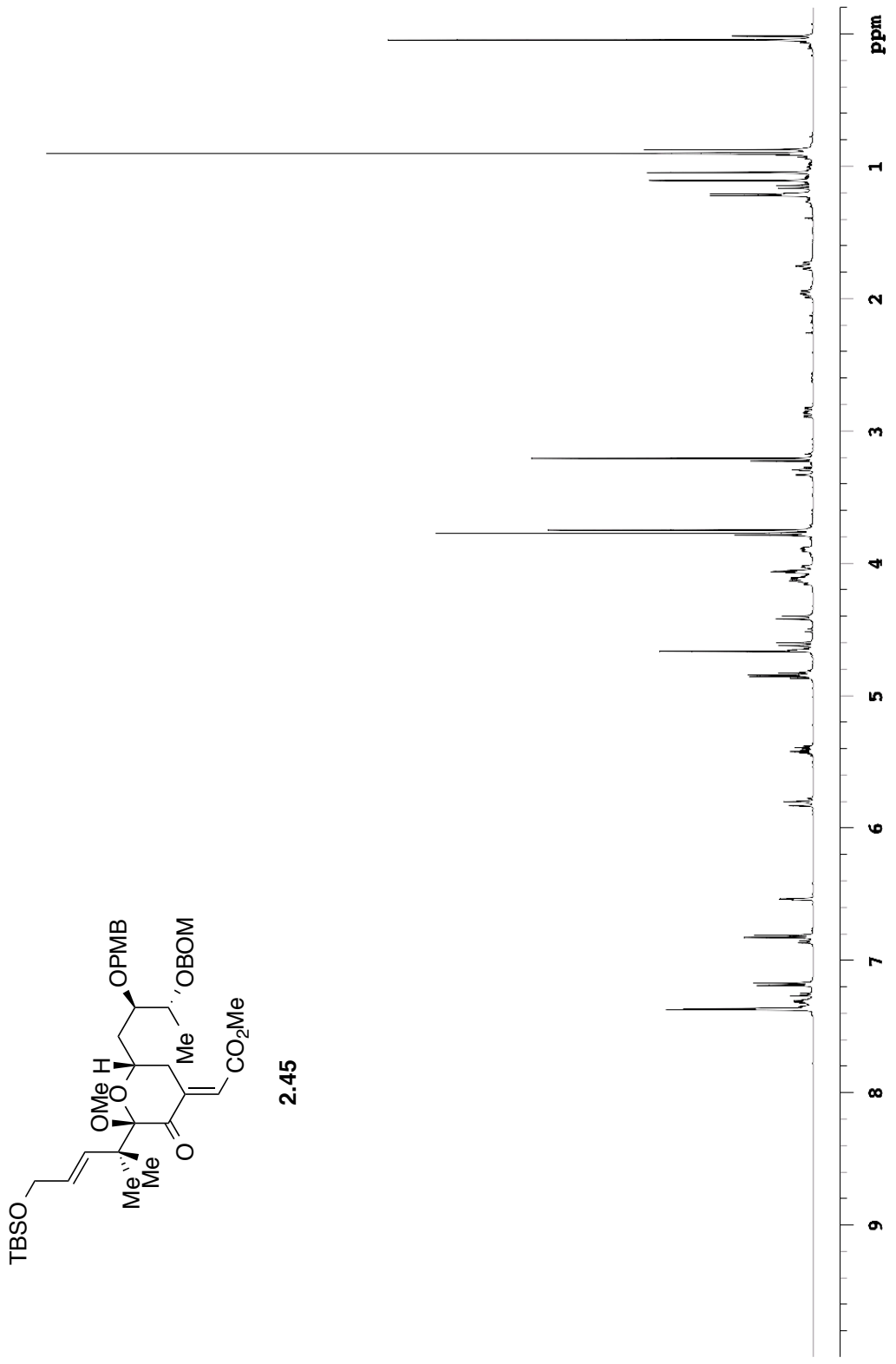


2.44

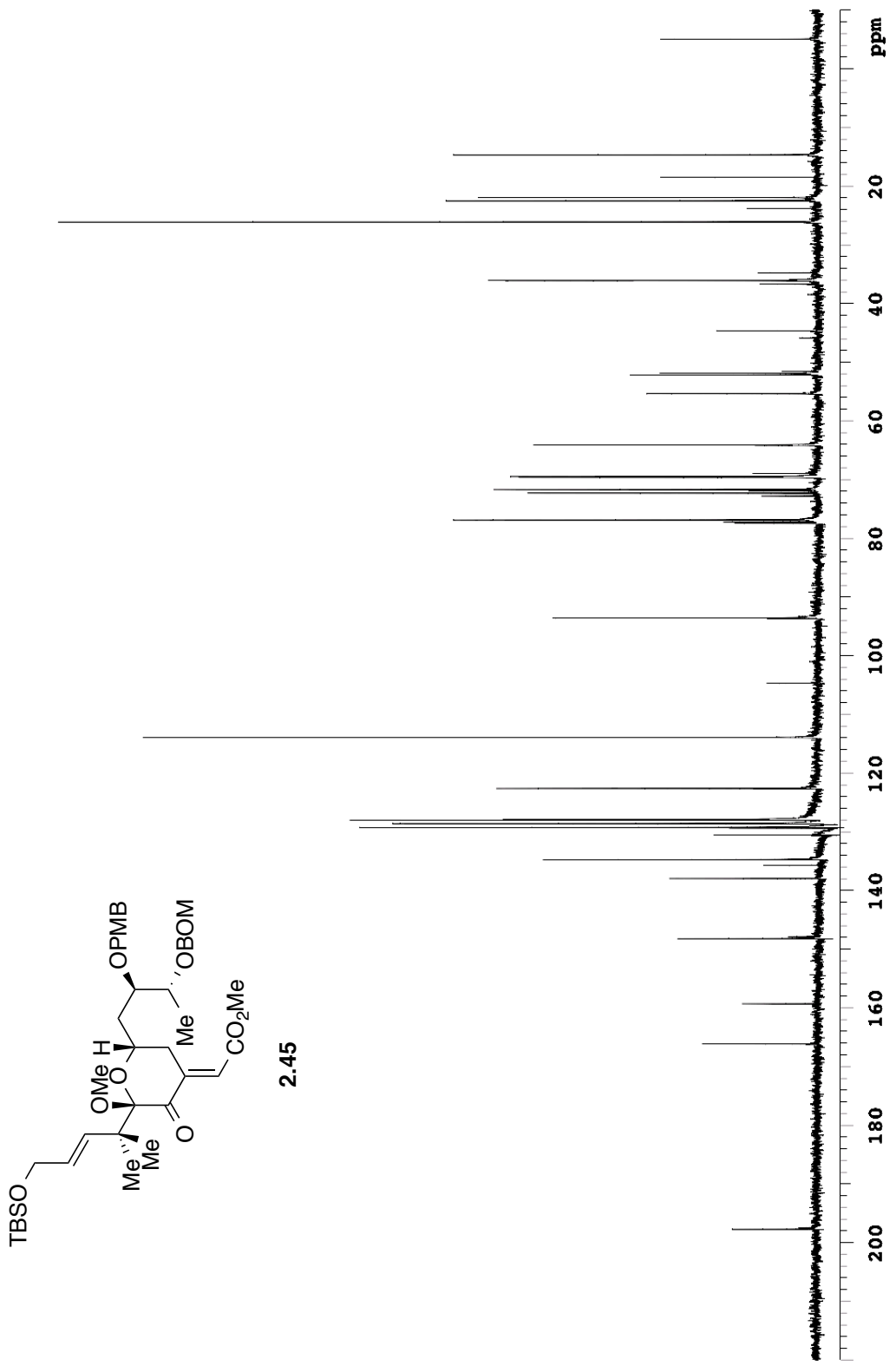

TBSO
Me
Me
O
H
Me
OPMB
OBOM
2.44
ppm
200
180
160
140
120
100
80
60
40
20

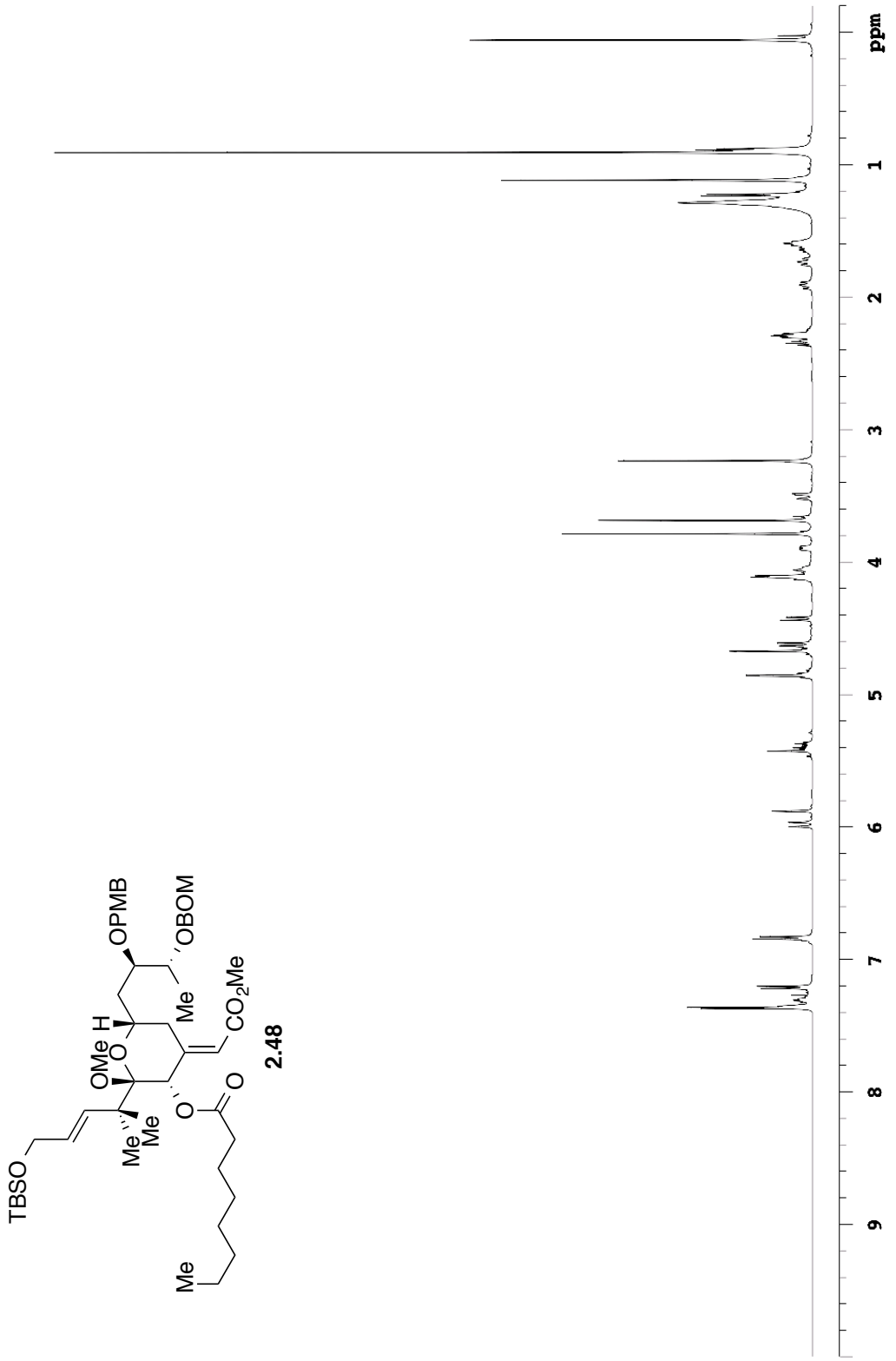

TBSO
Me
Me
OMe H
O
O
OPMB
Me
OBOM
2.20
ppm
1
2
3
4
5
6
7
8
9

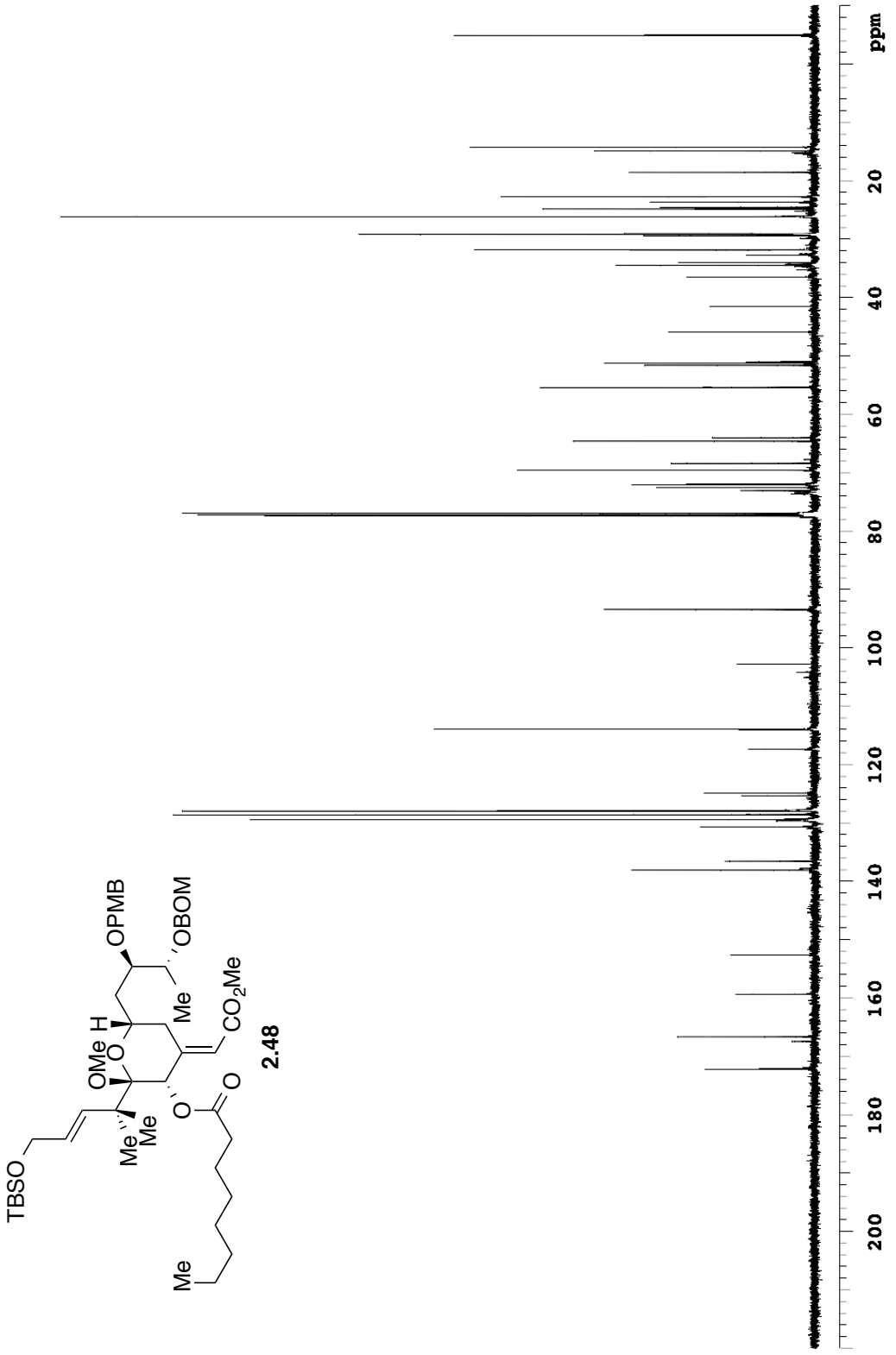

TBSO
Me
Me
OMe
H
O
O
Me
OPMB
OBOM
2.20
ppm
20
40
60
80
100
120
140
160
180
200

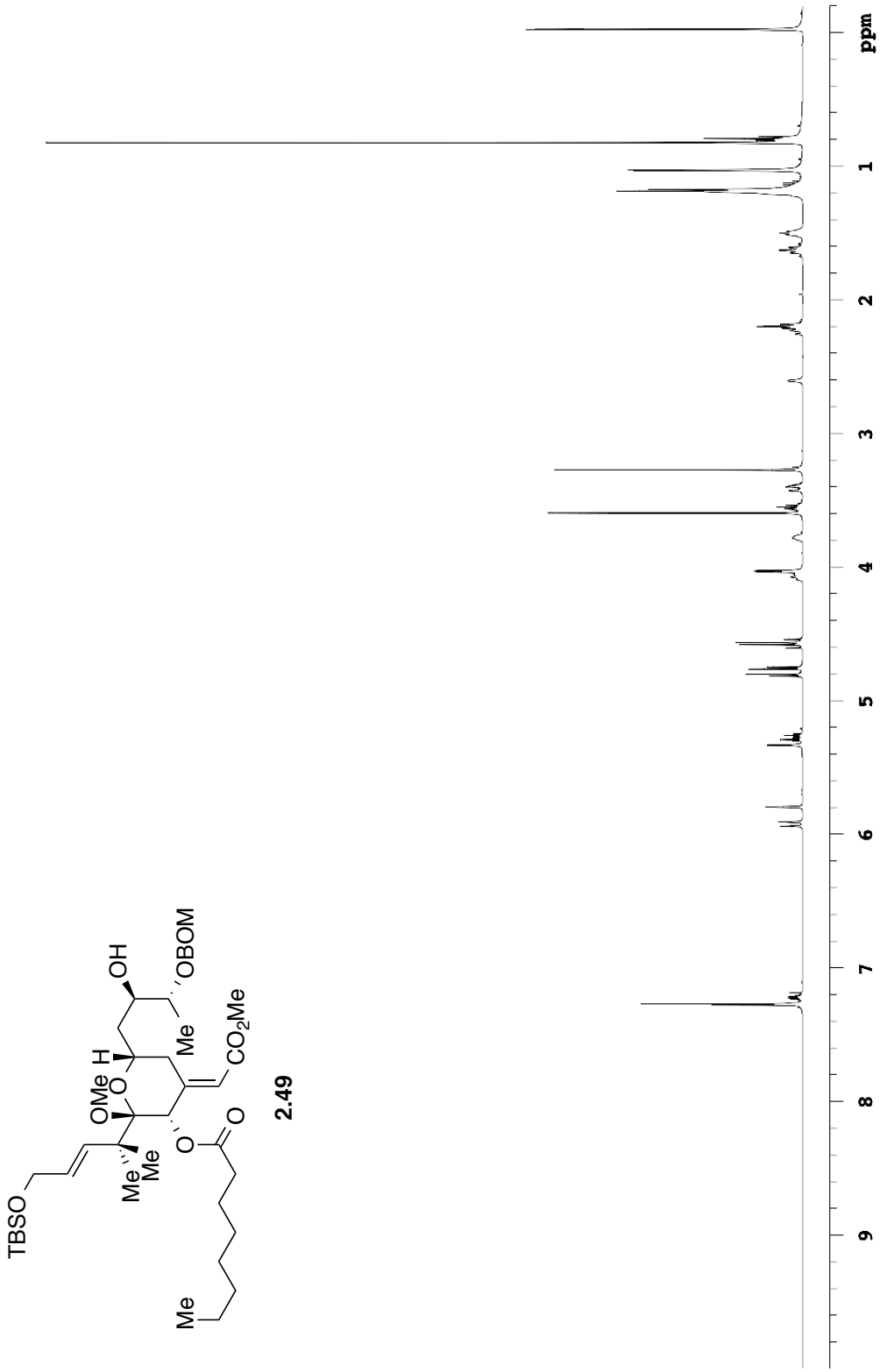


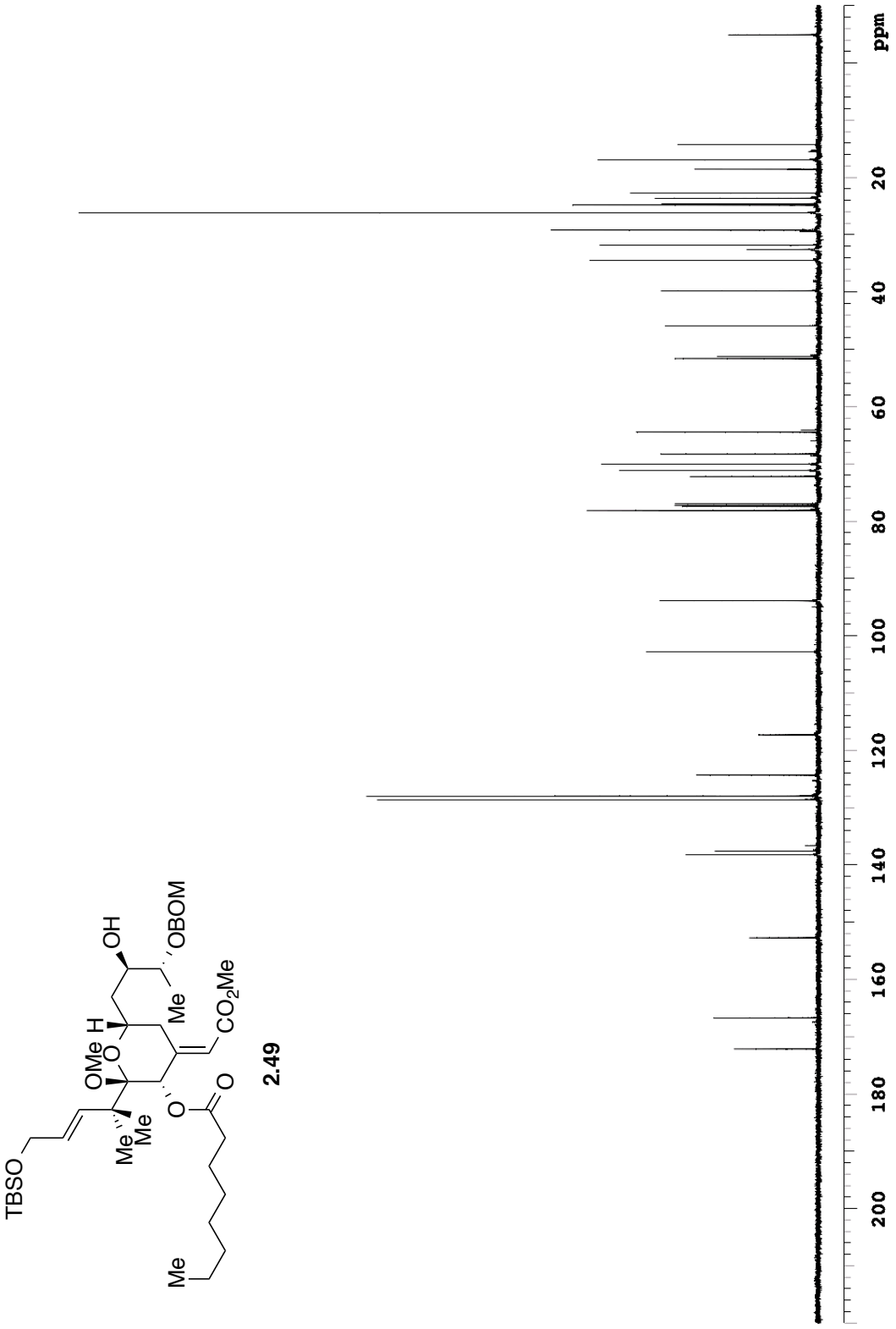
2.45

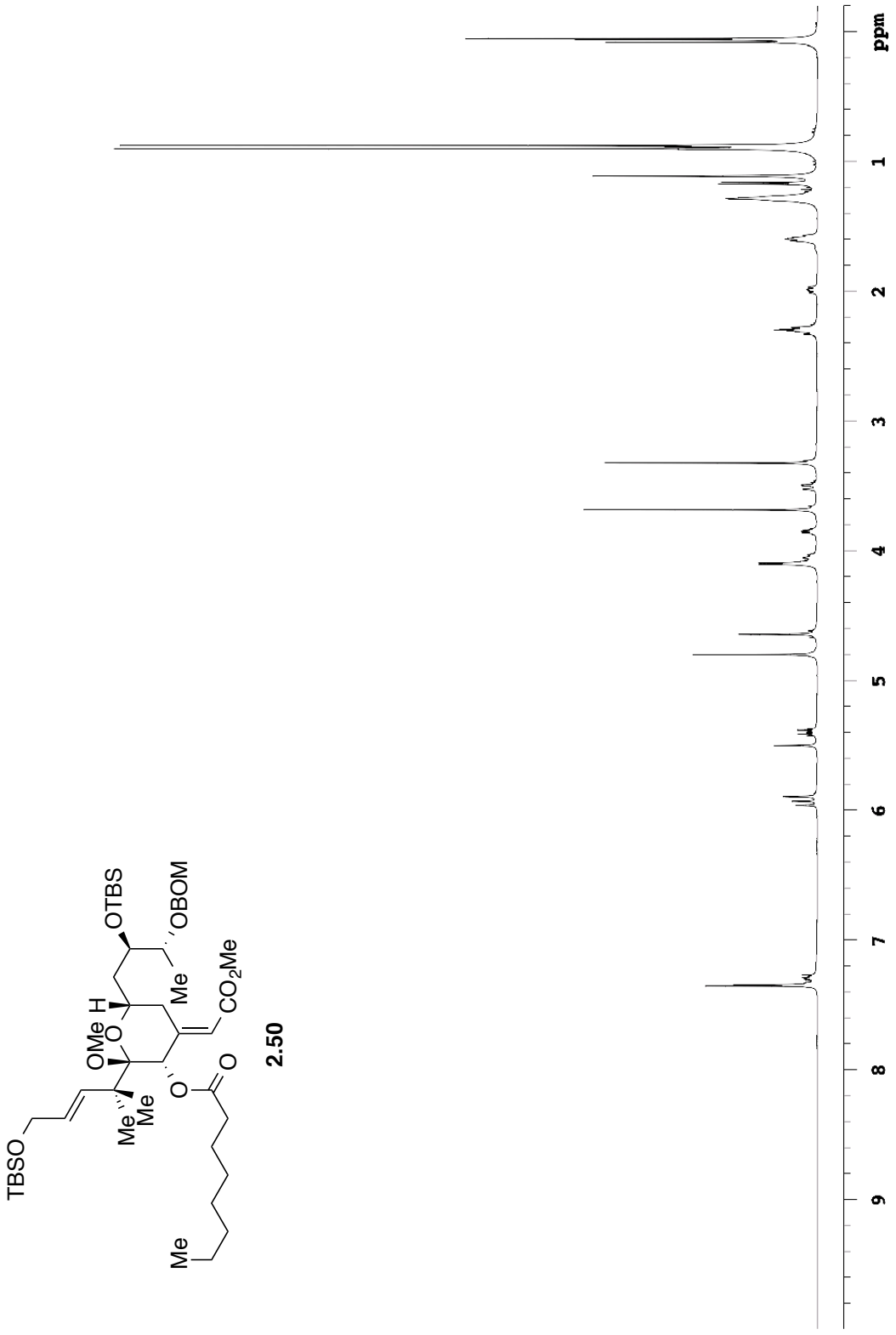

TBSO
Me
Me
OMe H
O
OPMB
Me
OBOM
O
CO2Me
2.45
ppm
200
180
160
140
120
100
80
60
40
20

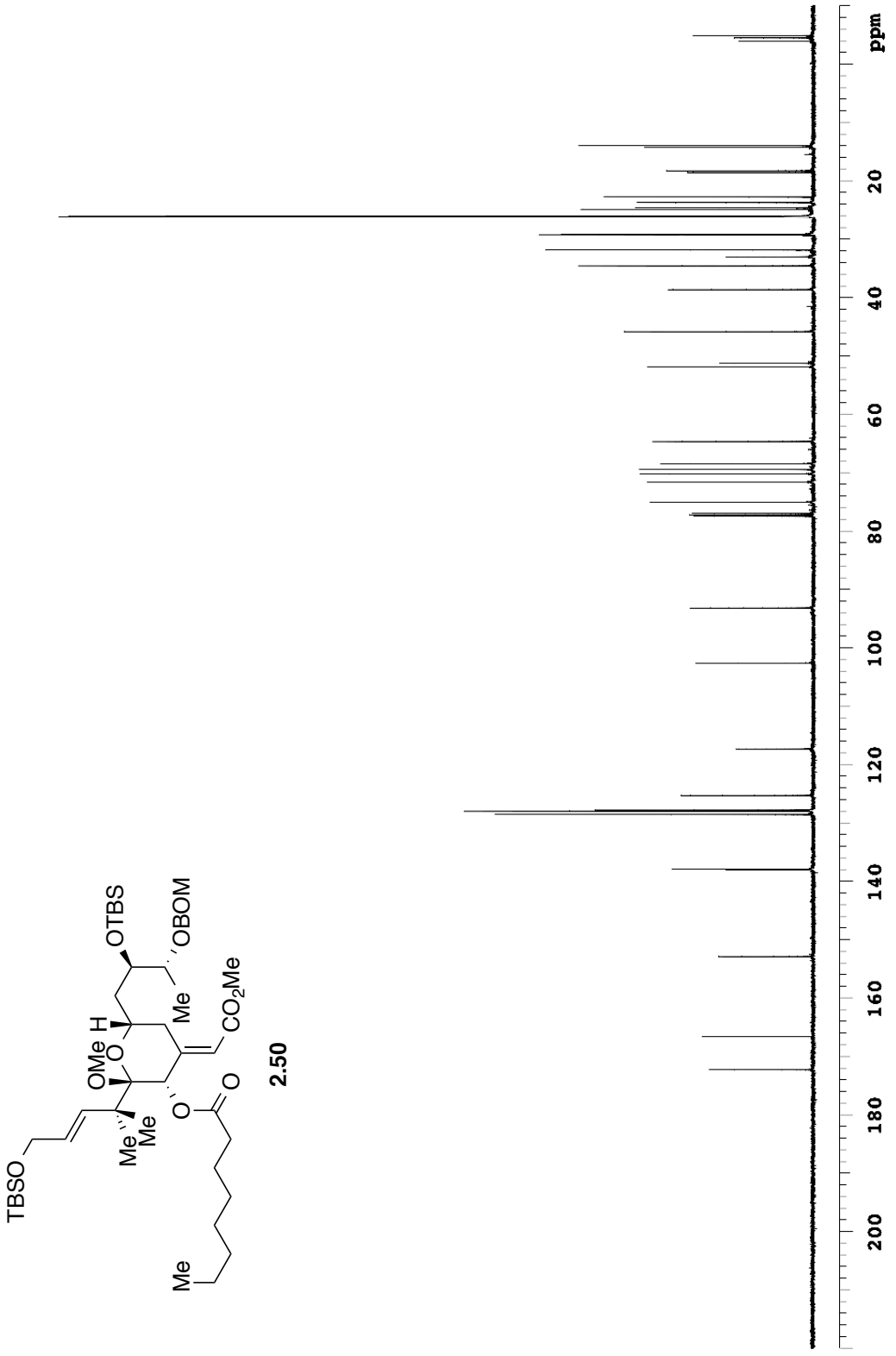

TBSO
OMe
H
O
OPMB
Me
Me
O
Me
OBOM
CO2Me
O
O
Me
2.48
ppm
1
2
3
4
5
6
7
8
9

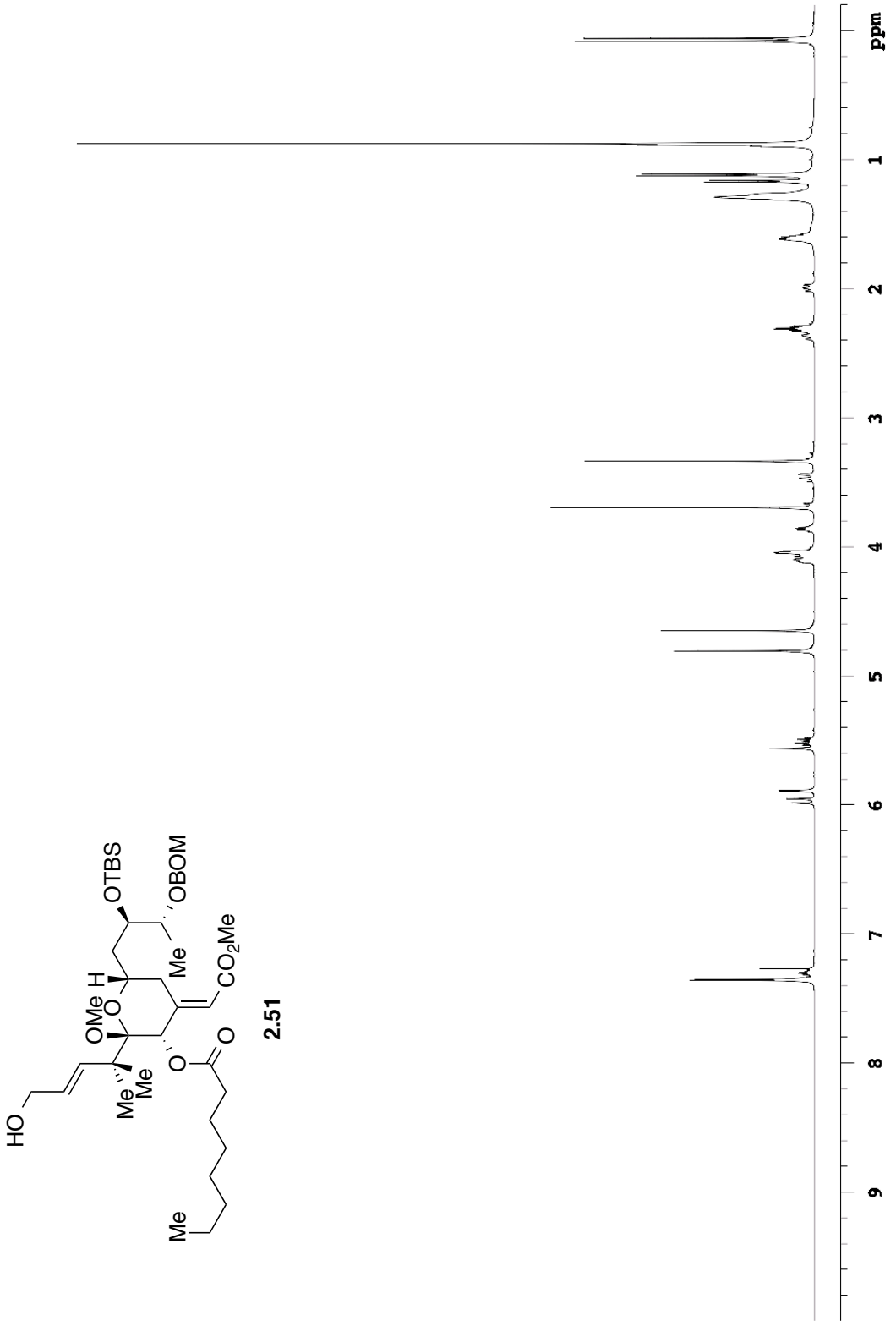

TBSO
OMe H
O
Me
Me
OPMB
OBOM
Me
CO2Me
O
O
O
Me
2.48
ppm
200
180
160
140
120
100
80
60
40
20

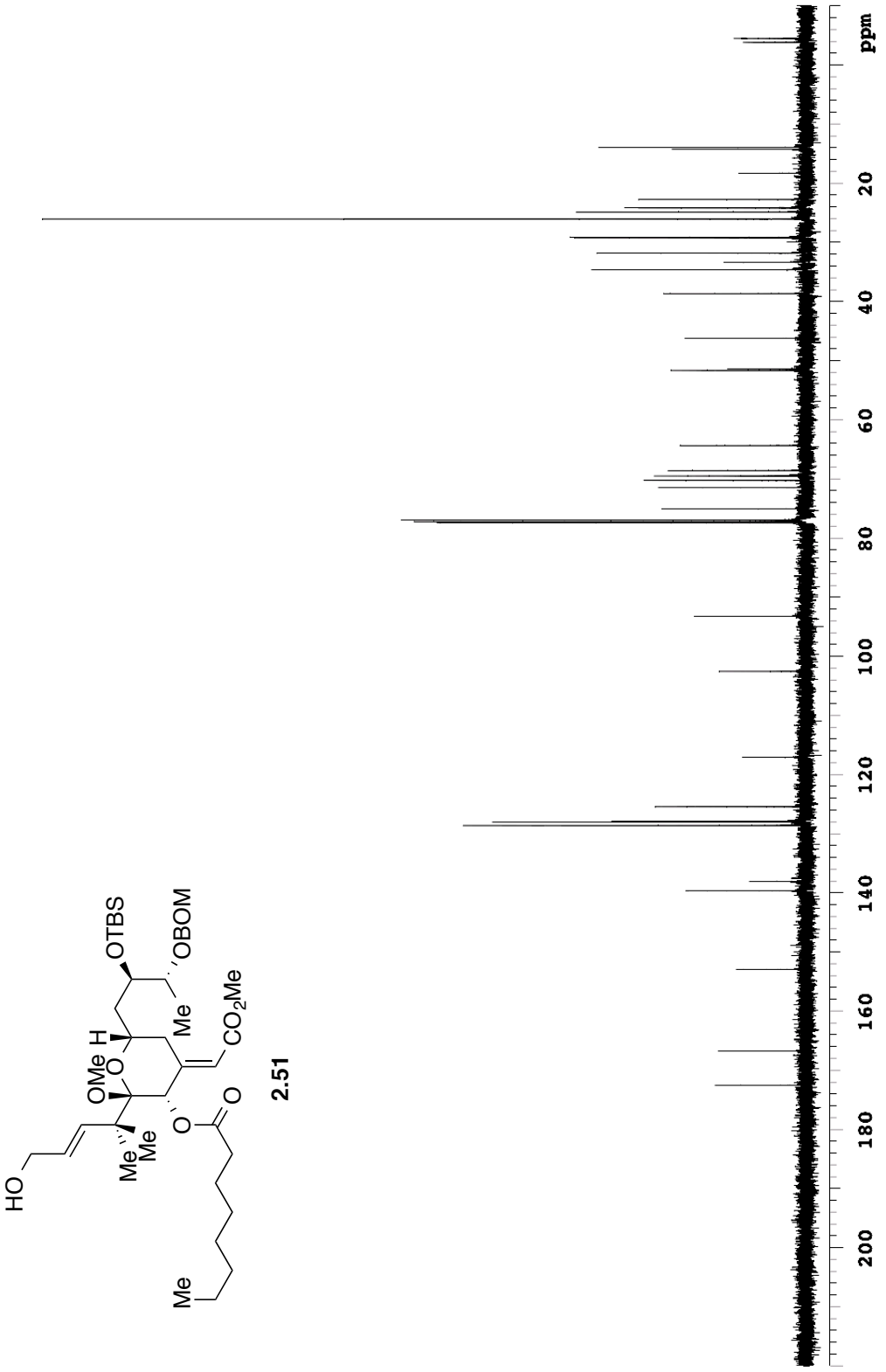

TBSO
Me
Me
OMe
H
O
OH
Me
OBOM
CO2Me
O
O
Me
2.49
ppm
1
2
3
4
5
6
7
8
9

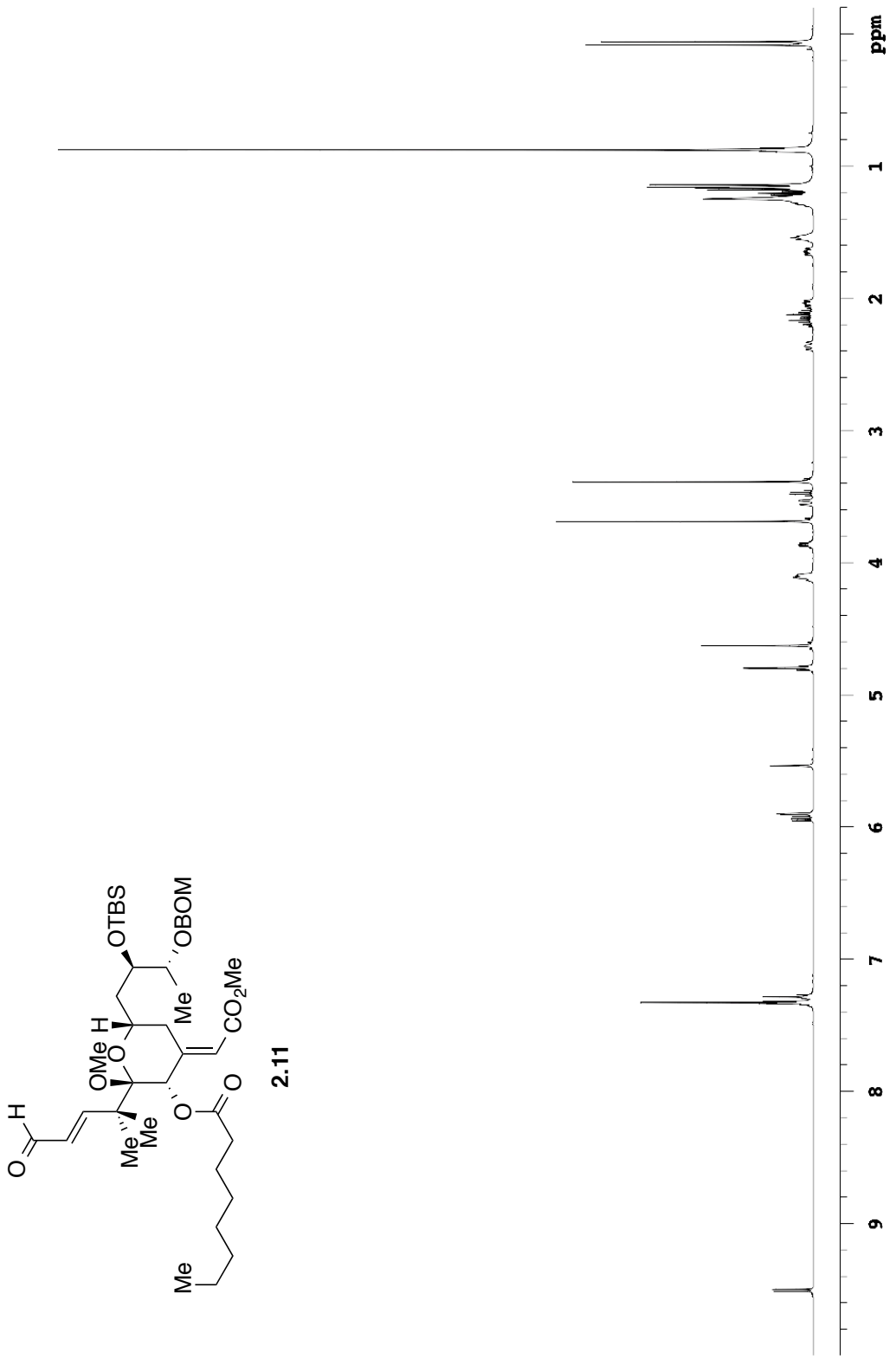

TBSO
OMe
H
O
OH
OBOM
Me
Me
Me
O
O
O
CO2Me
Me
2.49
ppm
200
180
160
140
120
100
80
60
40
20


TBSO
Me
Me
OMe H
O
O
OTBS
Me
OBOM
CO2Me
O
Me
2.50
ppm
1
2
3
4
5
6
7
8
9

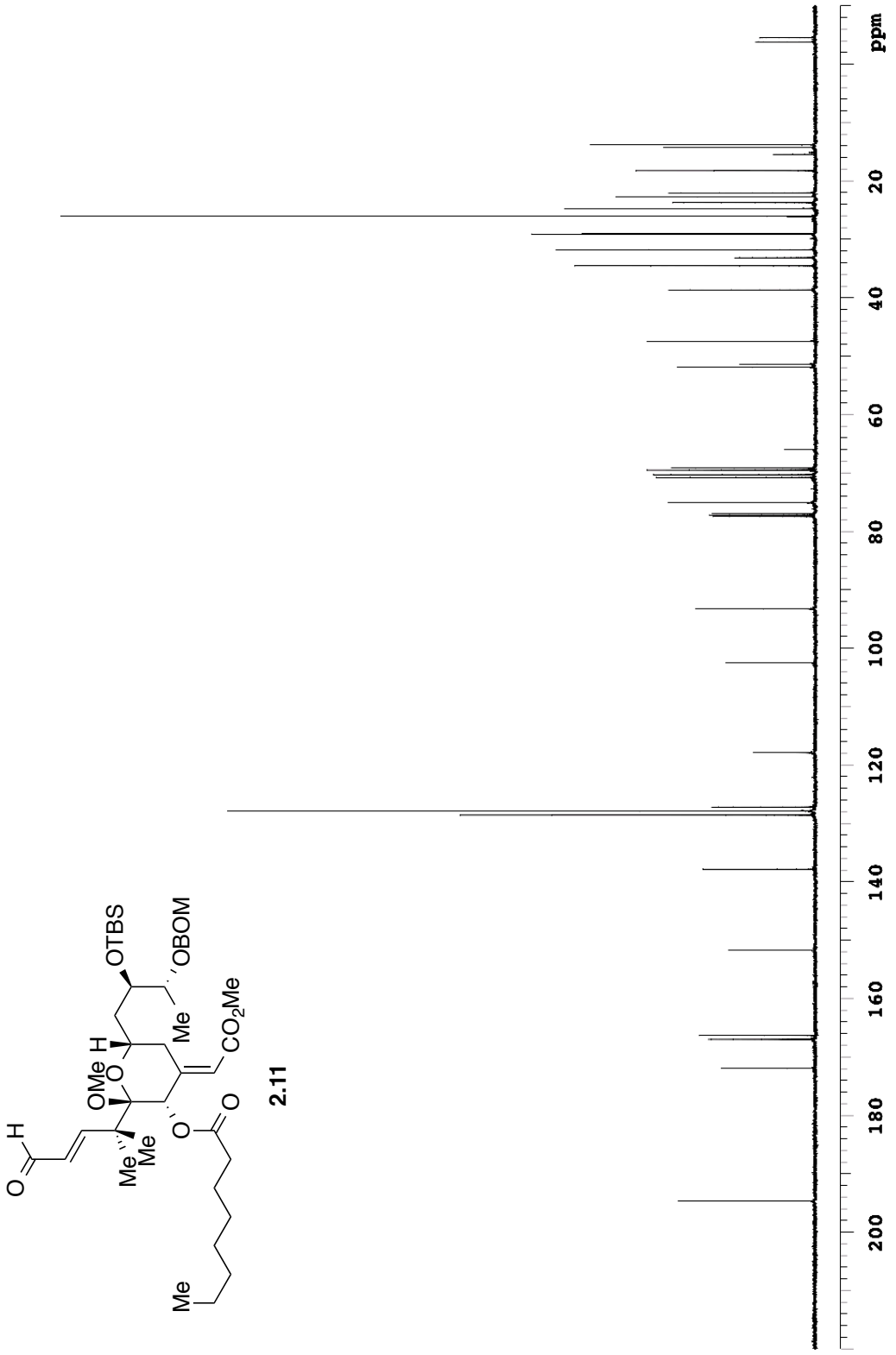

TBSO
OMe H
O
Me
Me
O
O
Me
OTBS
OBOM
CO2Me
2.50
ppm
200
180
160
140
120
100
80
60
40
20

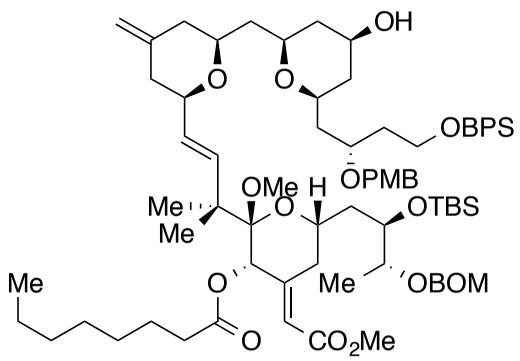

HO
Me
Me
OMe
H
O
O
OTBS
Me
OBOM
CO2Me
O
O
Me
2.51
ppm
1
2
3
4
5
6
7
8
9


HO
Me
Me
OMe H
O
OTBS
Me
OBOM
CO2Me
O
O
Me
2.51
ppm
200
180
160
140
120
100
80
60
40
20



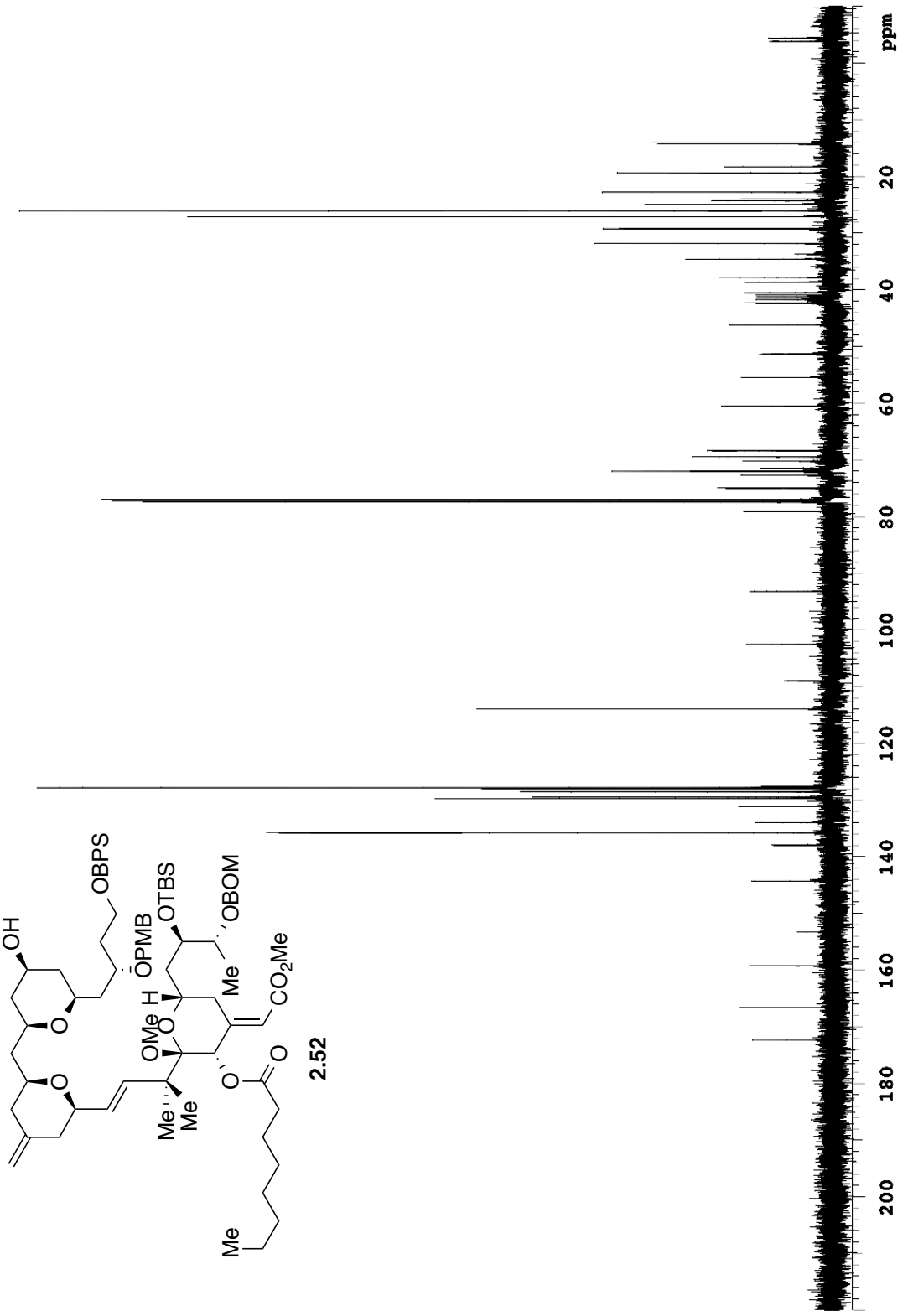
2.11

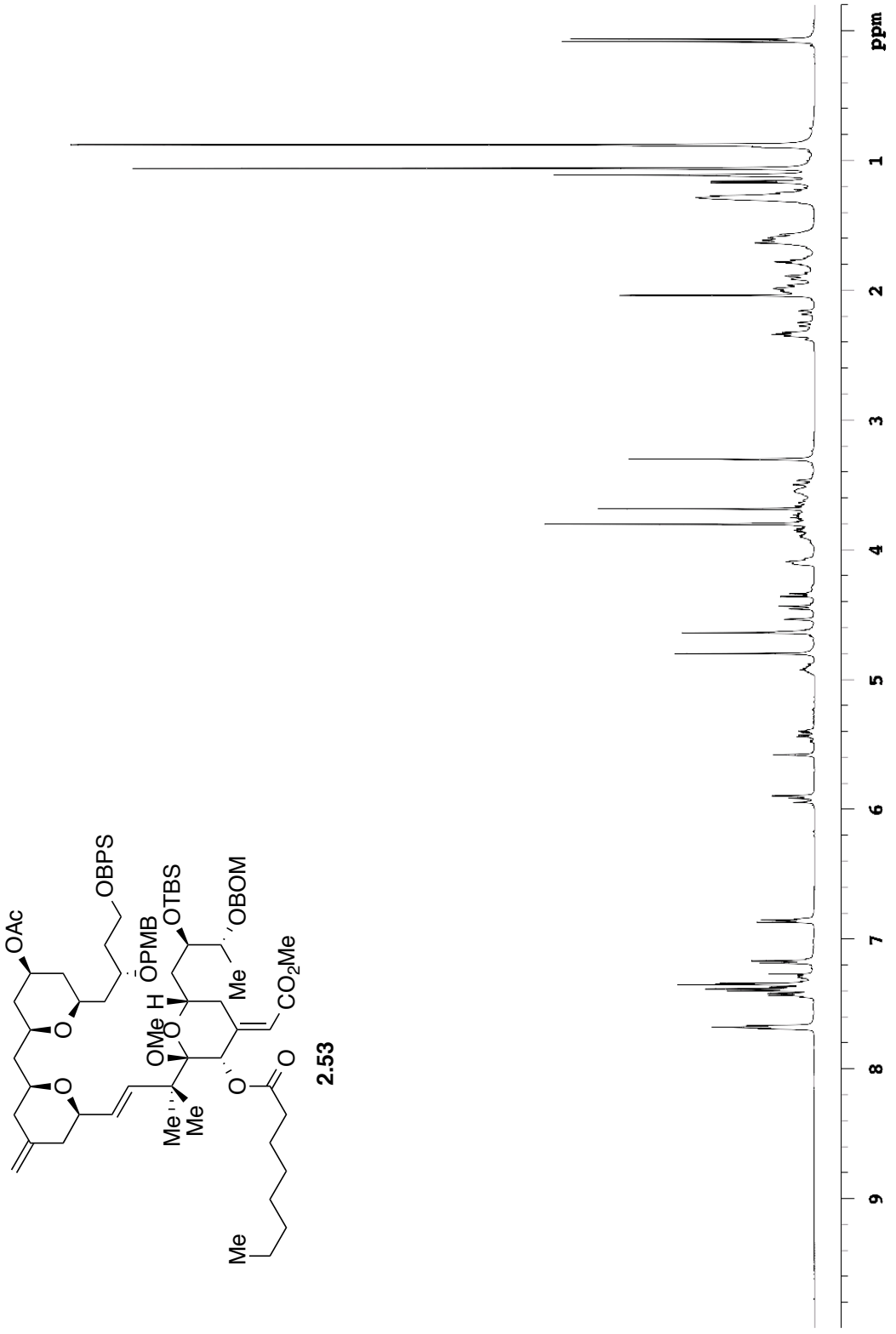

OTBS
OBOM
Me
CO2Me
OMe
H
O
Me
Me
O
O
O
Me
H
O
2.11
ppm
200
180
160
140
120
100
80
60
40
20


OH
O
O
OBPS
OPMB
OMe
H
Me
Me
O
OTBS
Me
O
Me
OBOM
O
CO2Me

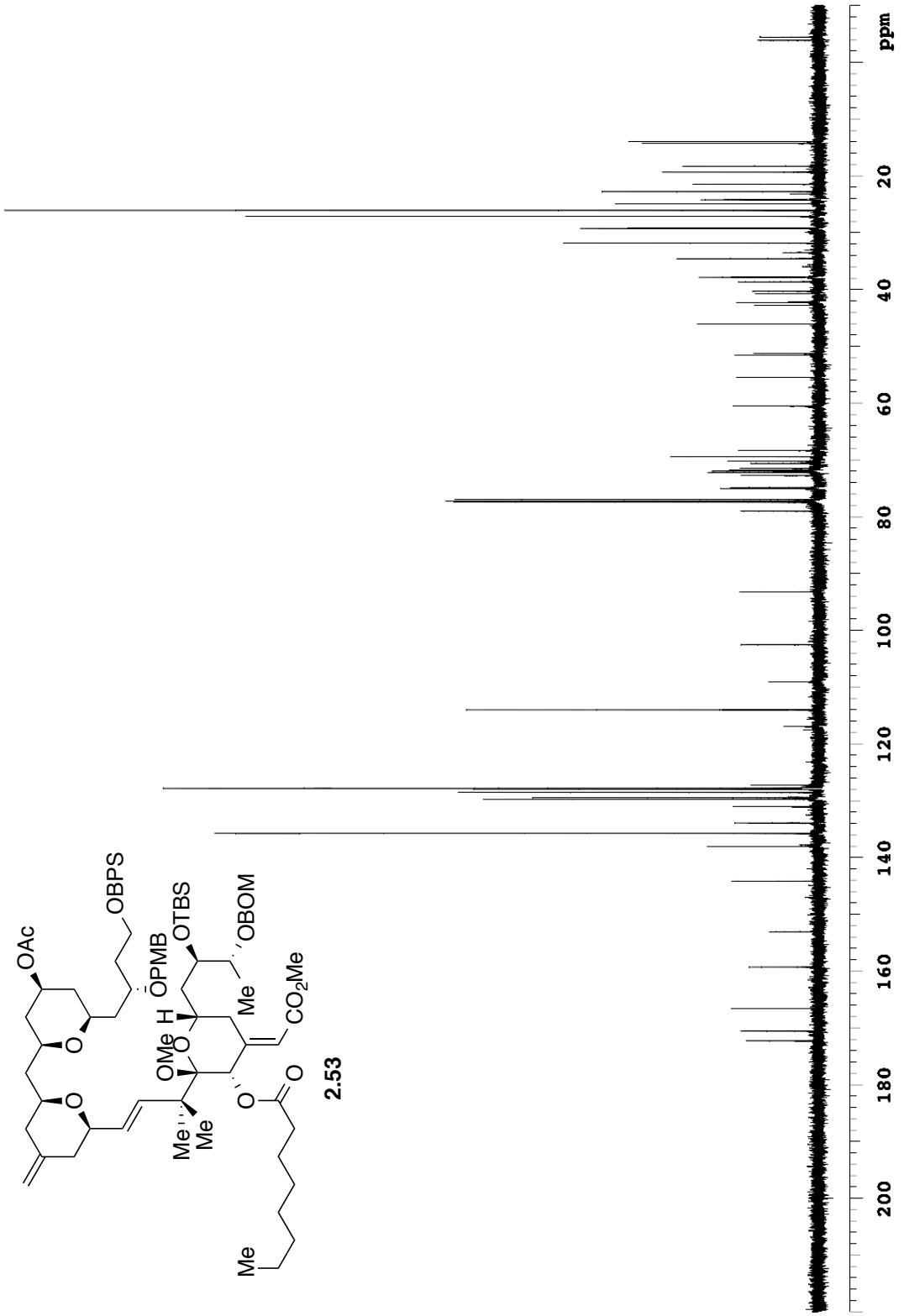
2.52

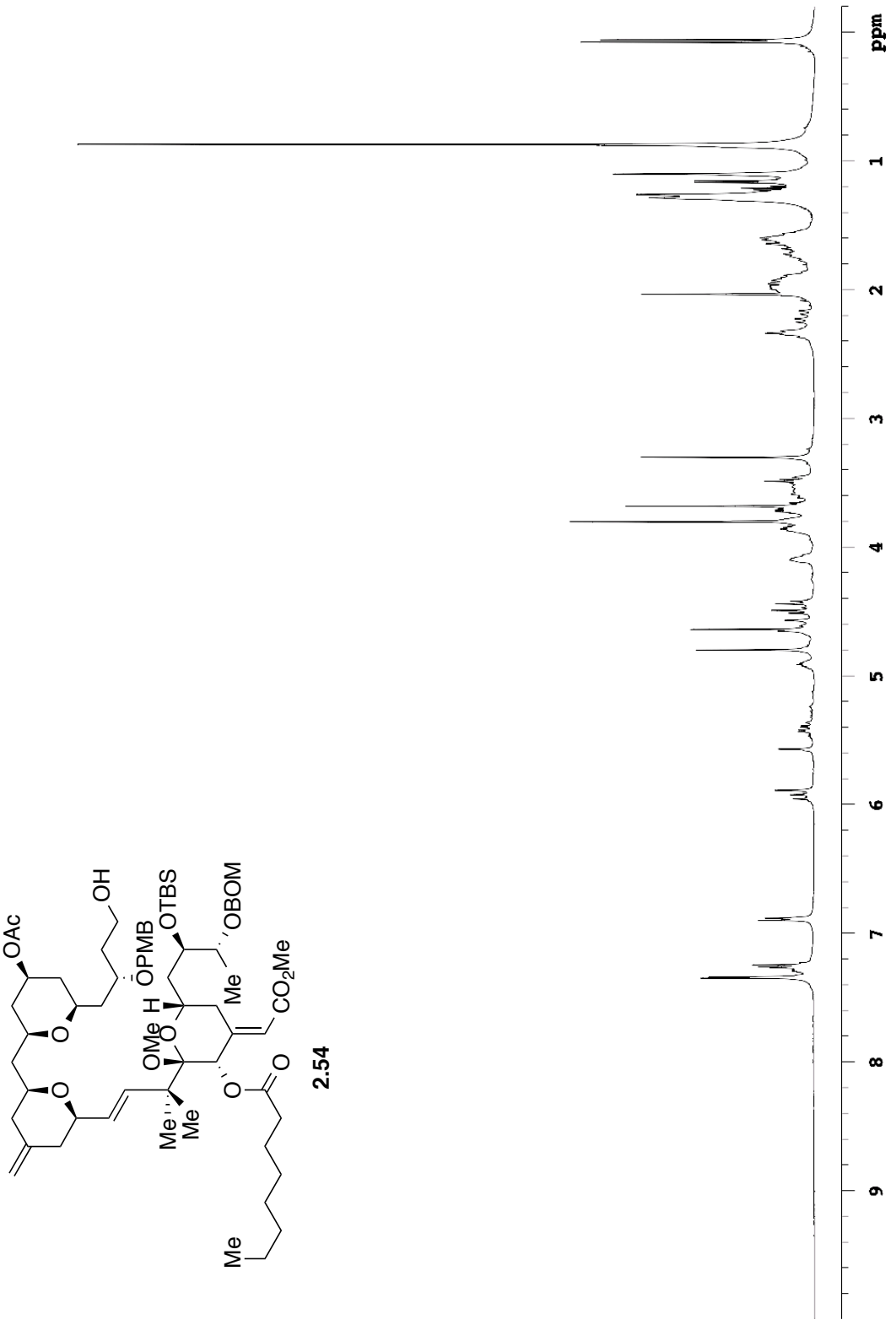
9
8
7
6
5
4
3
2
1
ppm

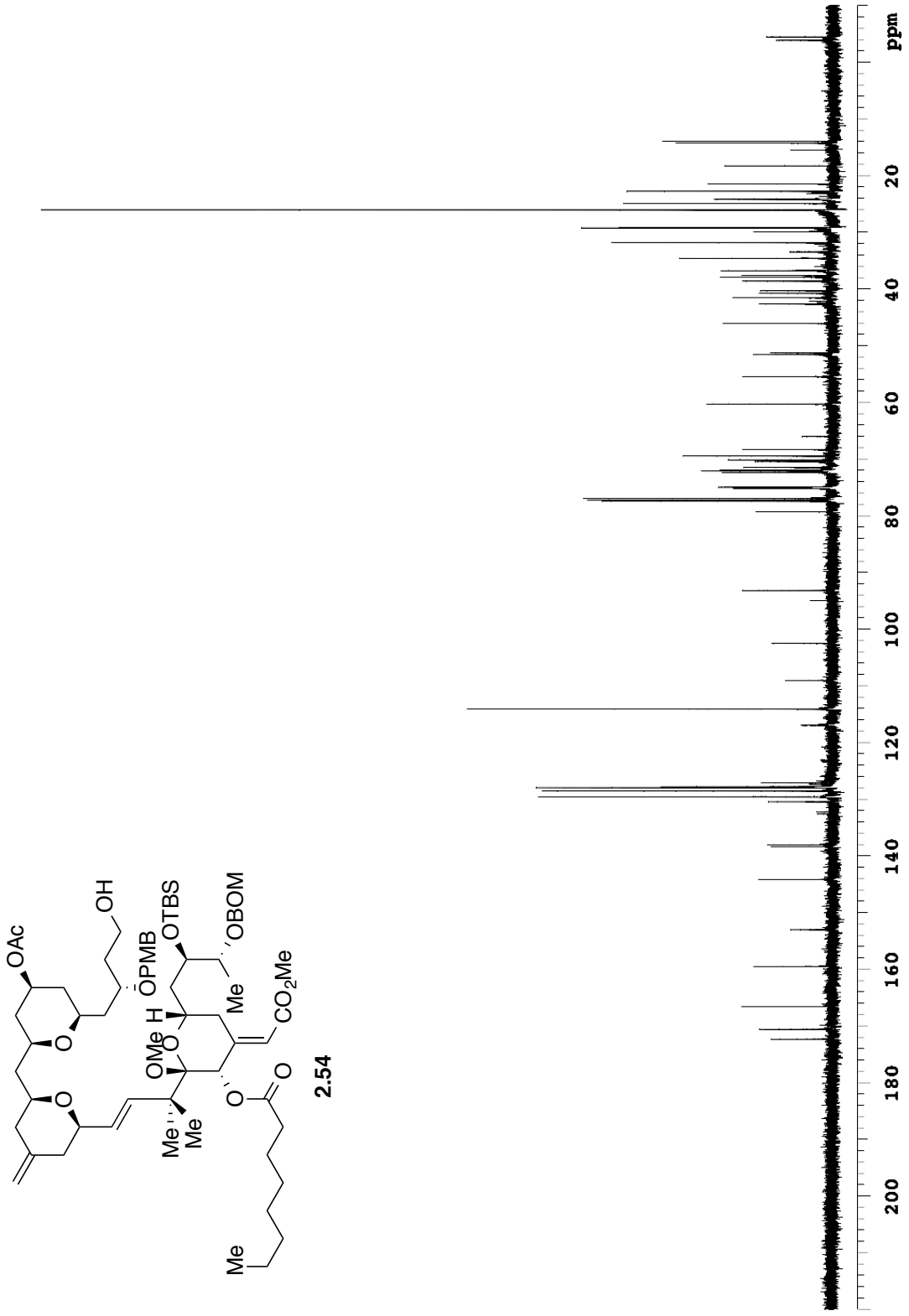

OH
OBPS
OPMB
OTBS
OBOM
OMe
H
Me
Me
Me
CO2Me
Me
2.52
ppm
20
40
60
80
100
120
140
160
180
200


OAc
OBPS
OPMB
OTBS
OBOM
OMe
H
Me
Me
Me
CO2Me
Me
O
O
O
O
O
O
ppm
1
2
3
4
5
6
7
8
9

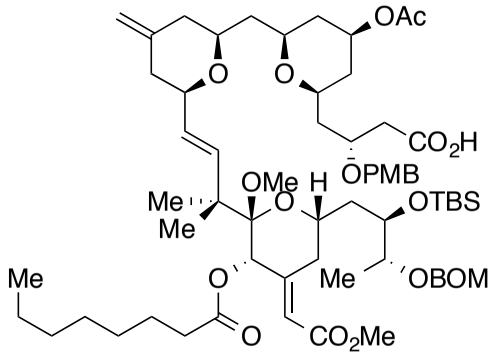
2.53


OAc
OBPS
ŌPMB
OTBS
OMe
H
O
Me
Me
Me
OBOM
CO2Me
O
O
O
Me
2.53
ppm
200
180
160
140
120
100
80
60
40
20


OAc
OH
OPMB
OTBS
OBOM
OMe
H
Me
Me
Me
CO2Me
Me
2.54
ppm
1
2
3
4
5
6
7
8
9

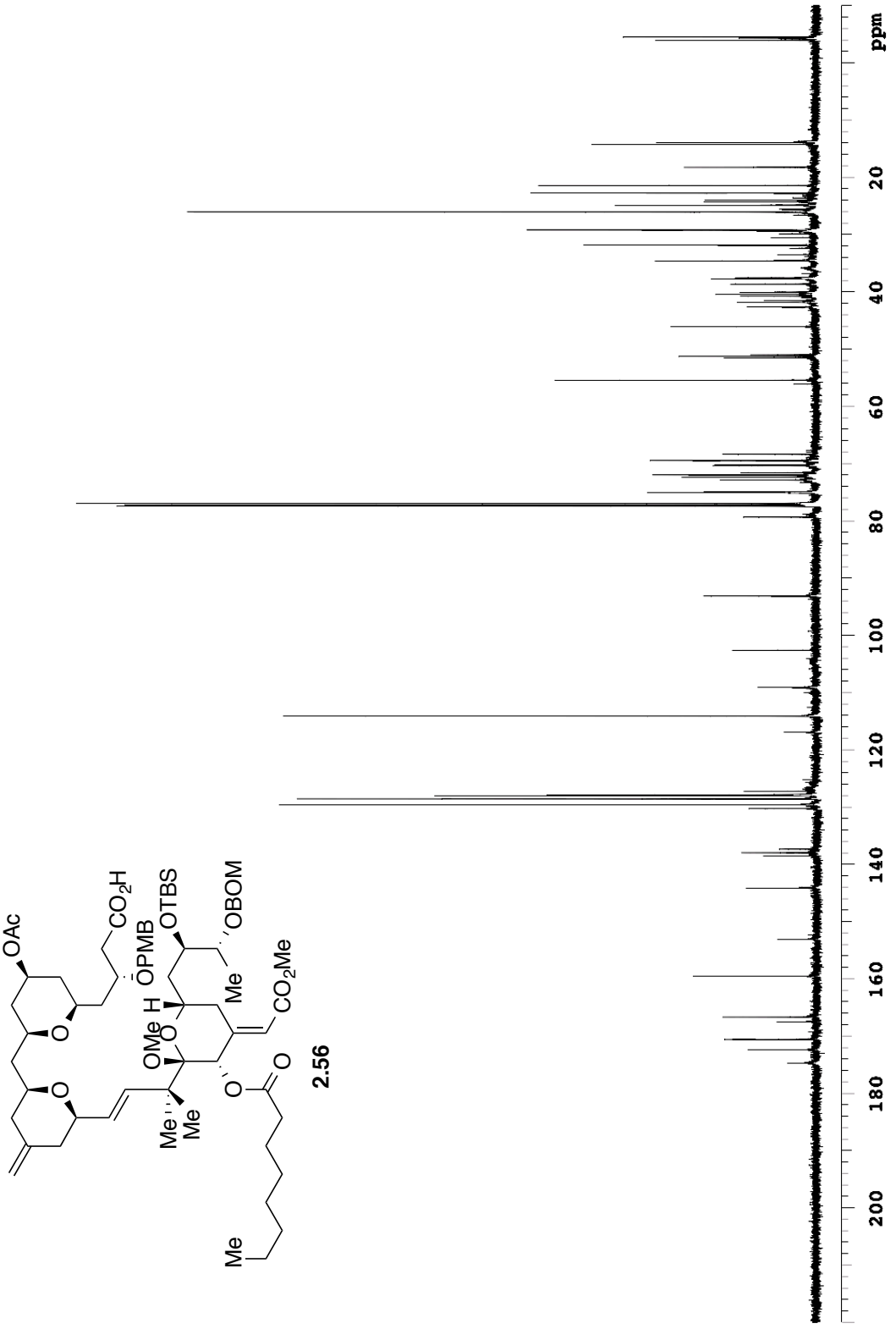

OAc
OH
OPMB
OTBS
OBOM
OMe
H
Me
Me
Me
CO2Me
O
O
O
O
O
Me
ppm
200
180
160
140
120
100
80
60
40
20

2.54

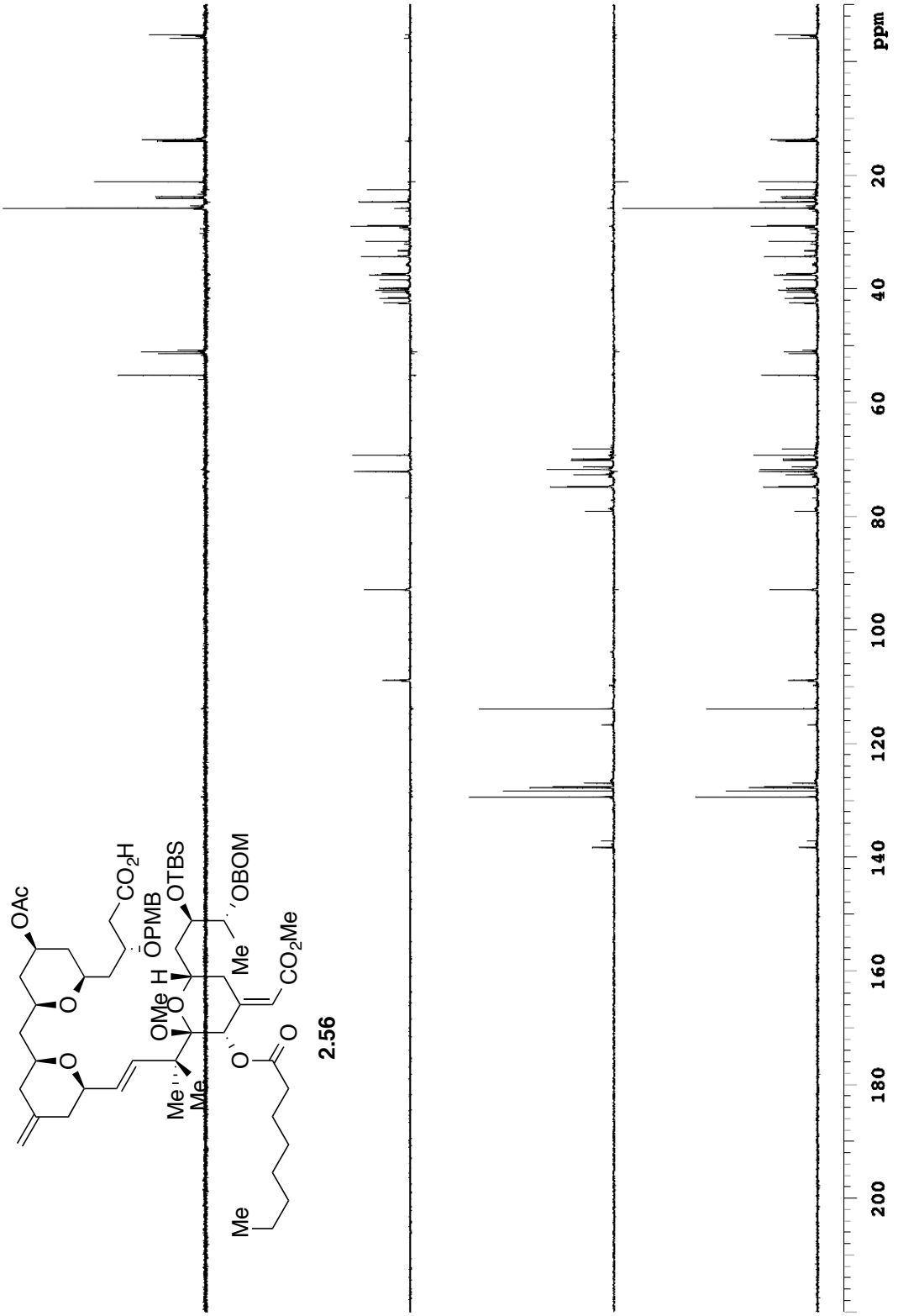

OAc
O
O
CO2H
OPMB
OMe
H
O
OTBS
Me
Me
Me
O
Me
OBOM
O
CO2Me

2.56

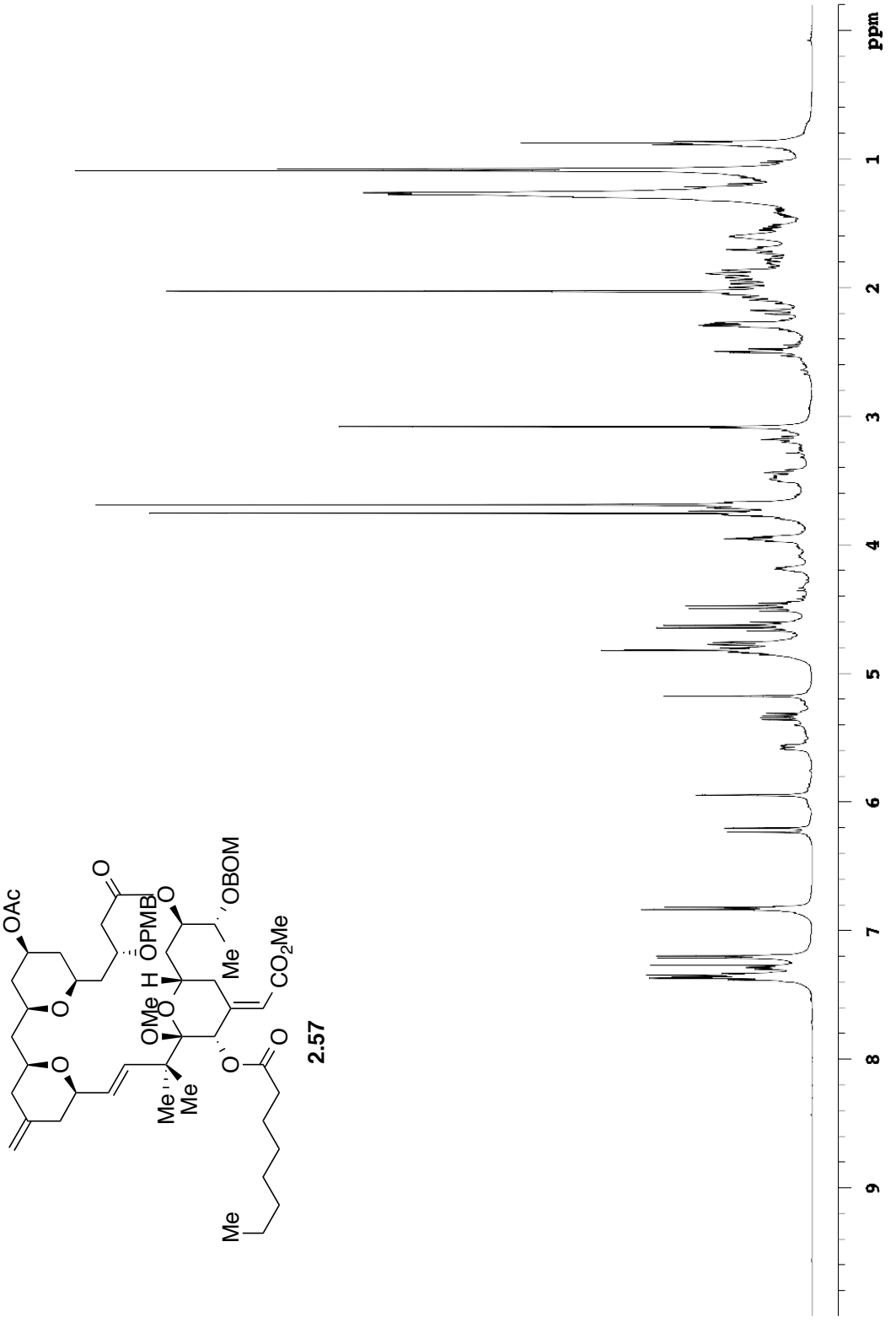
9
8
7
6
5
4
3
2
1
ppm

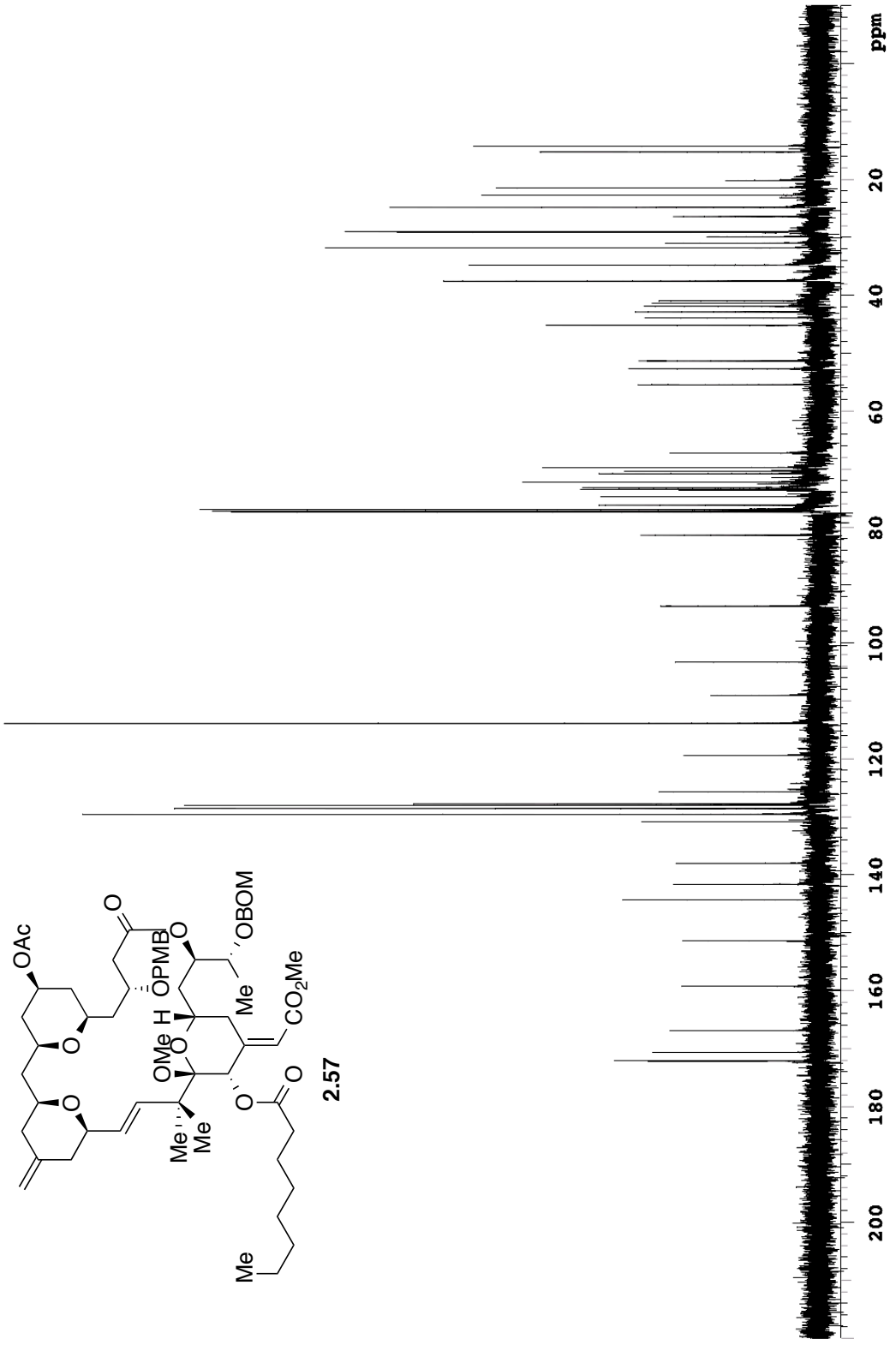

OAc
CO2H
OPMB
OTBS
OBOM
OMe H
Me
Me
Me
CO2Me
Me
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

2.56

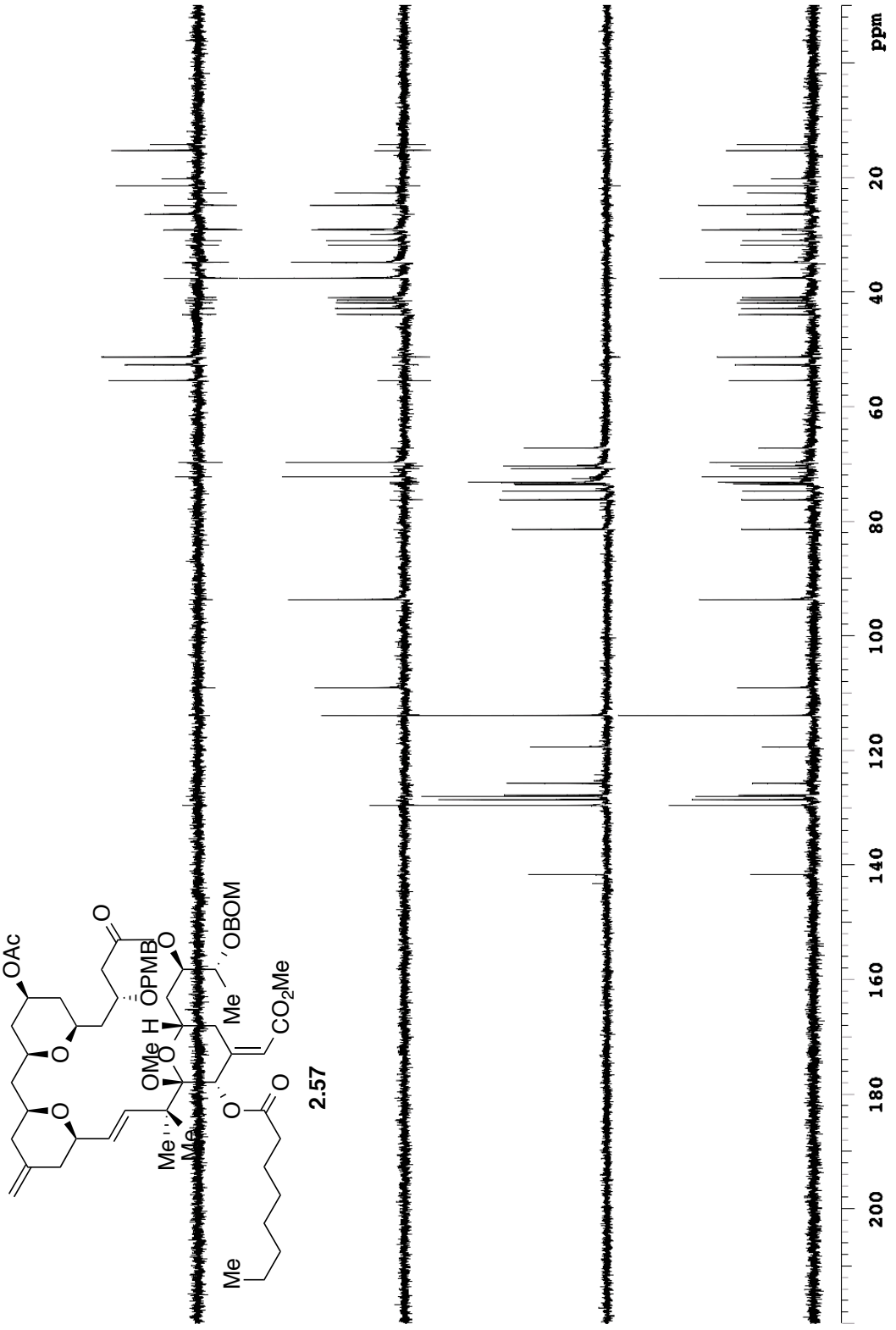

OAc
CO2H
OPMB
OTBS
OBOM
OMe H
Me
Me
Me
CO2Me
Me
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

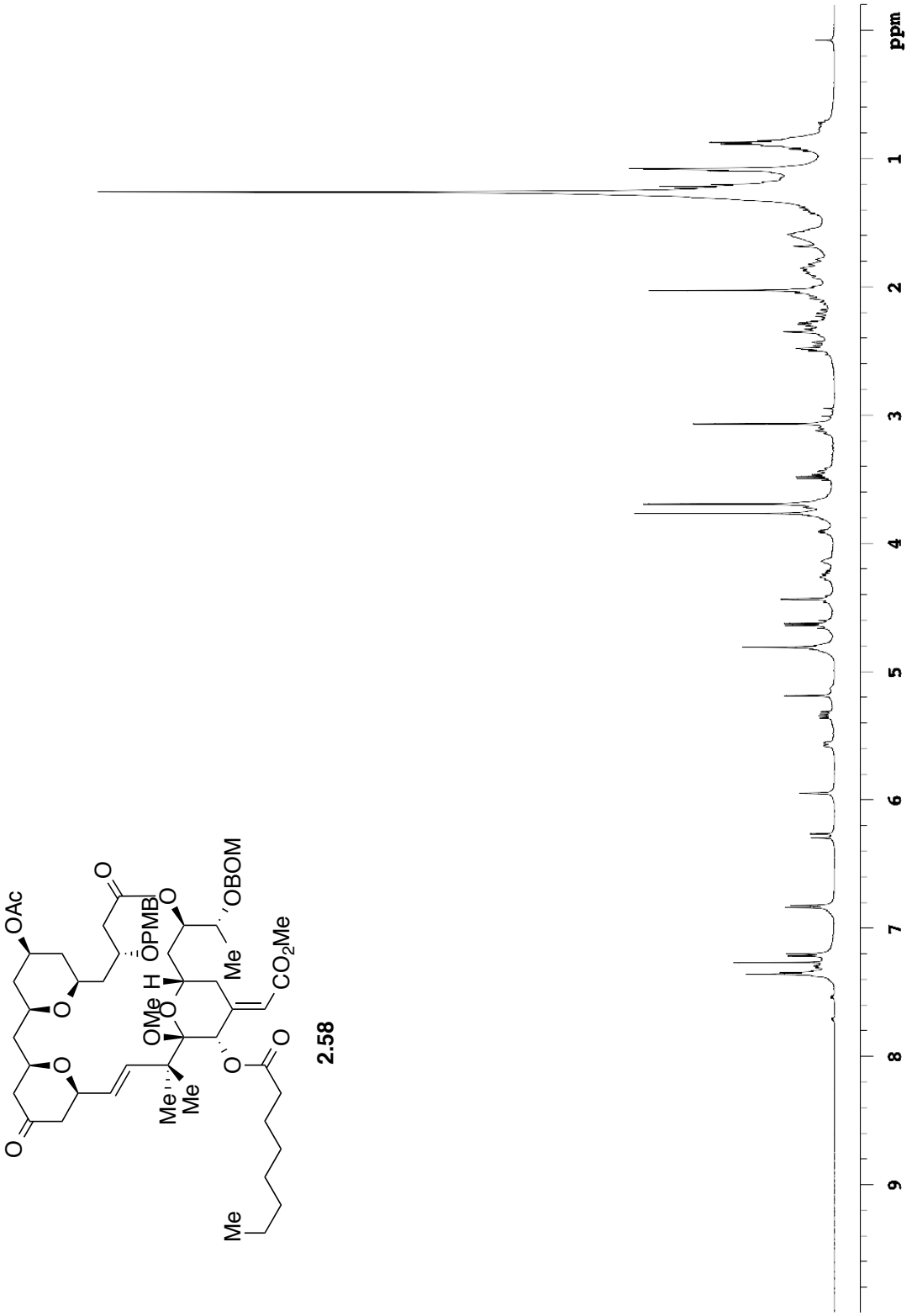
2.56


OAc
O
OPMB
H
OMe
O
O
O
Me
Me
O
O
Me
OBOM
Me
CO2Me
O
2.57
ppm
1
2
3
4
5
6
7
8
9

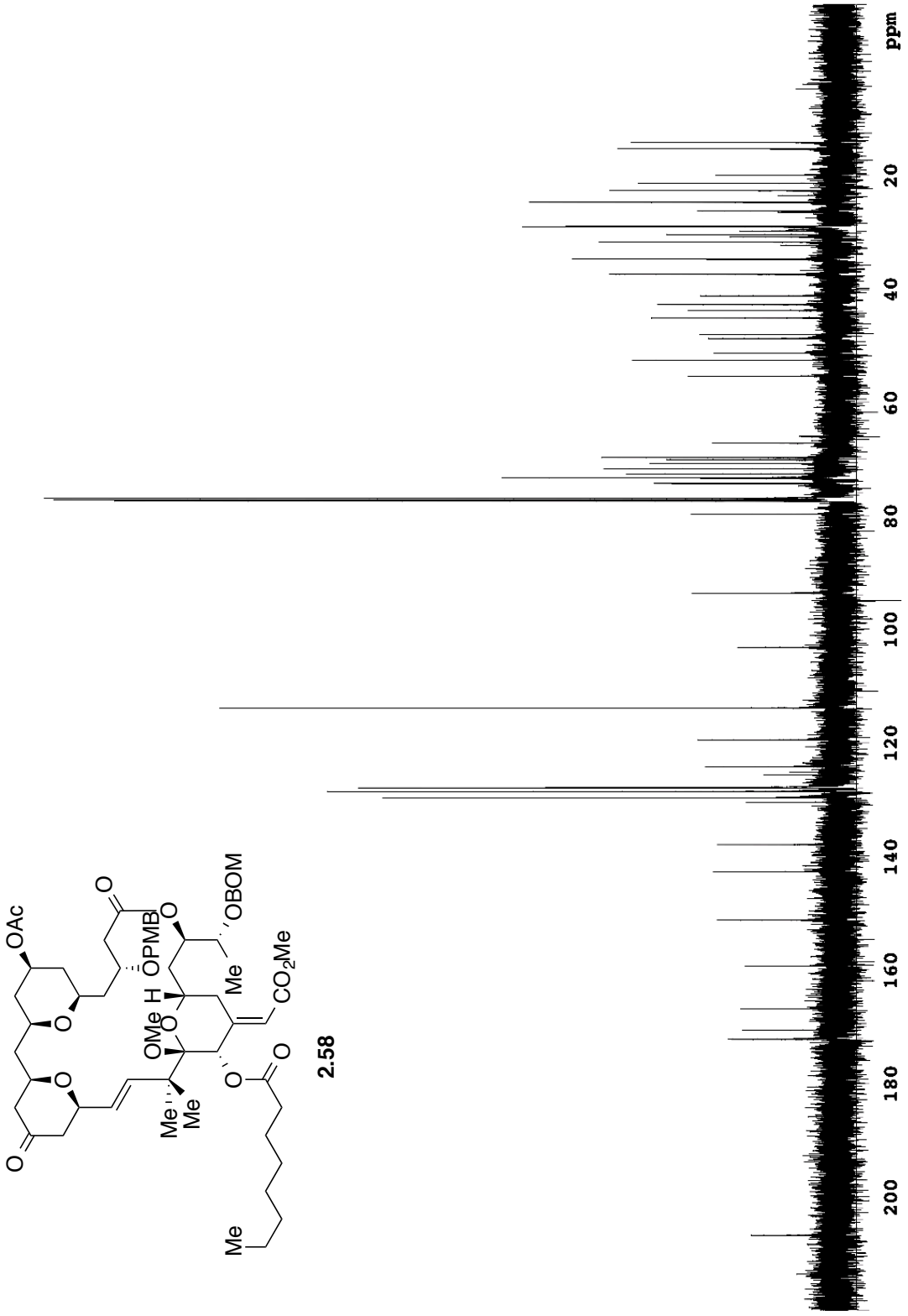

OAc
O
OPMB
OMe H
O
O
Me
Me
O
O
Me
OBOM
CO2Me
O
Me
ppm
200
180
160
140
120
100
80
60
40
20

2.57

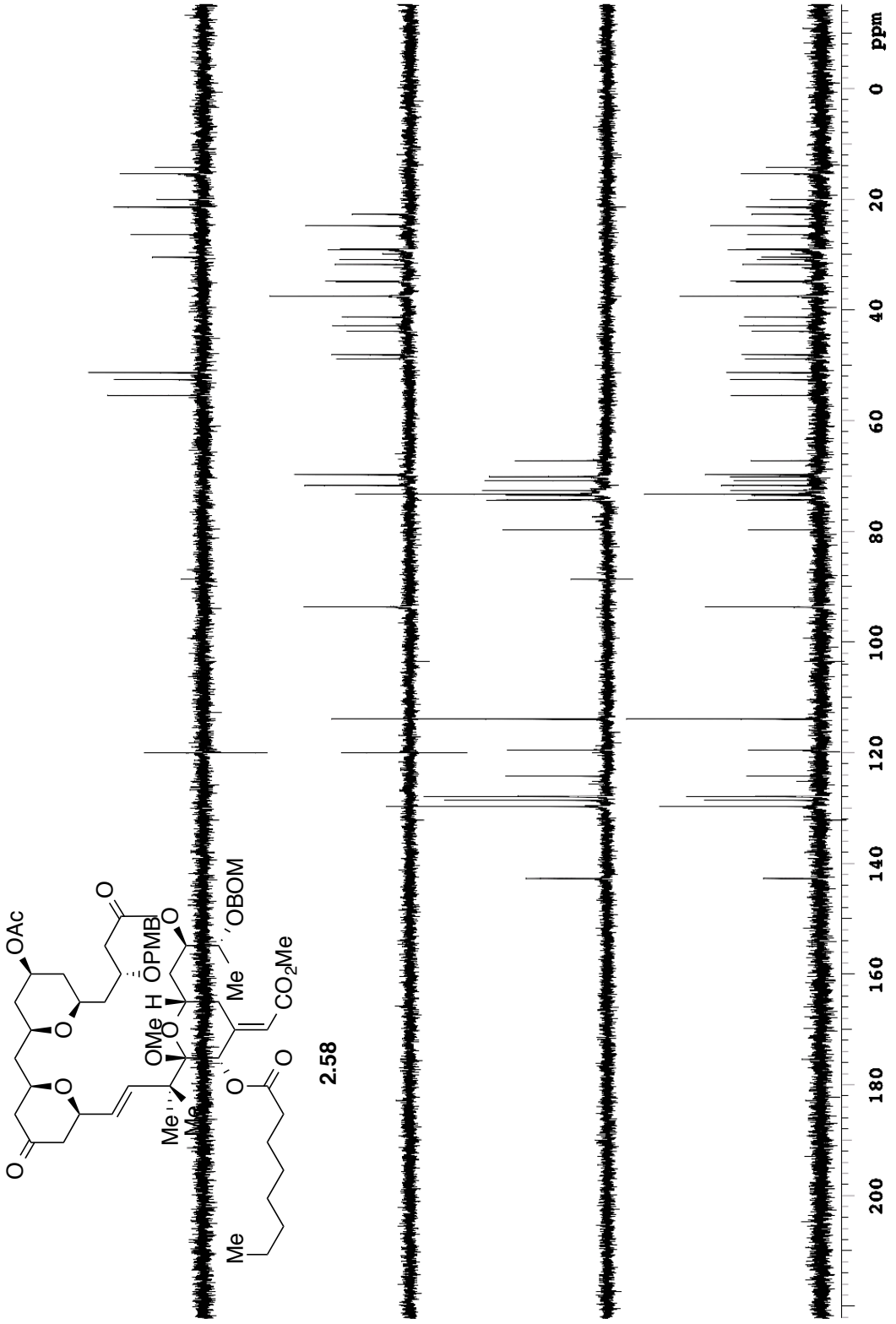

OAc
O
O
O
OMe H OPMB
O
O
Me
Me
Me
O
O
Me
OBOM
CO2Me
2.57
200
180
160
140
120
100
80
60
40
20
ppm

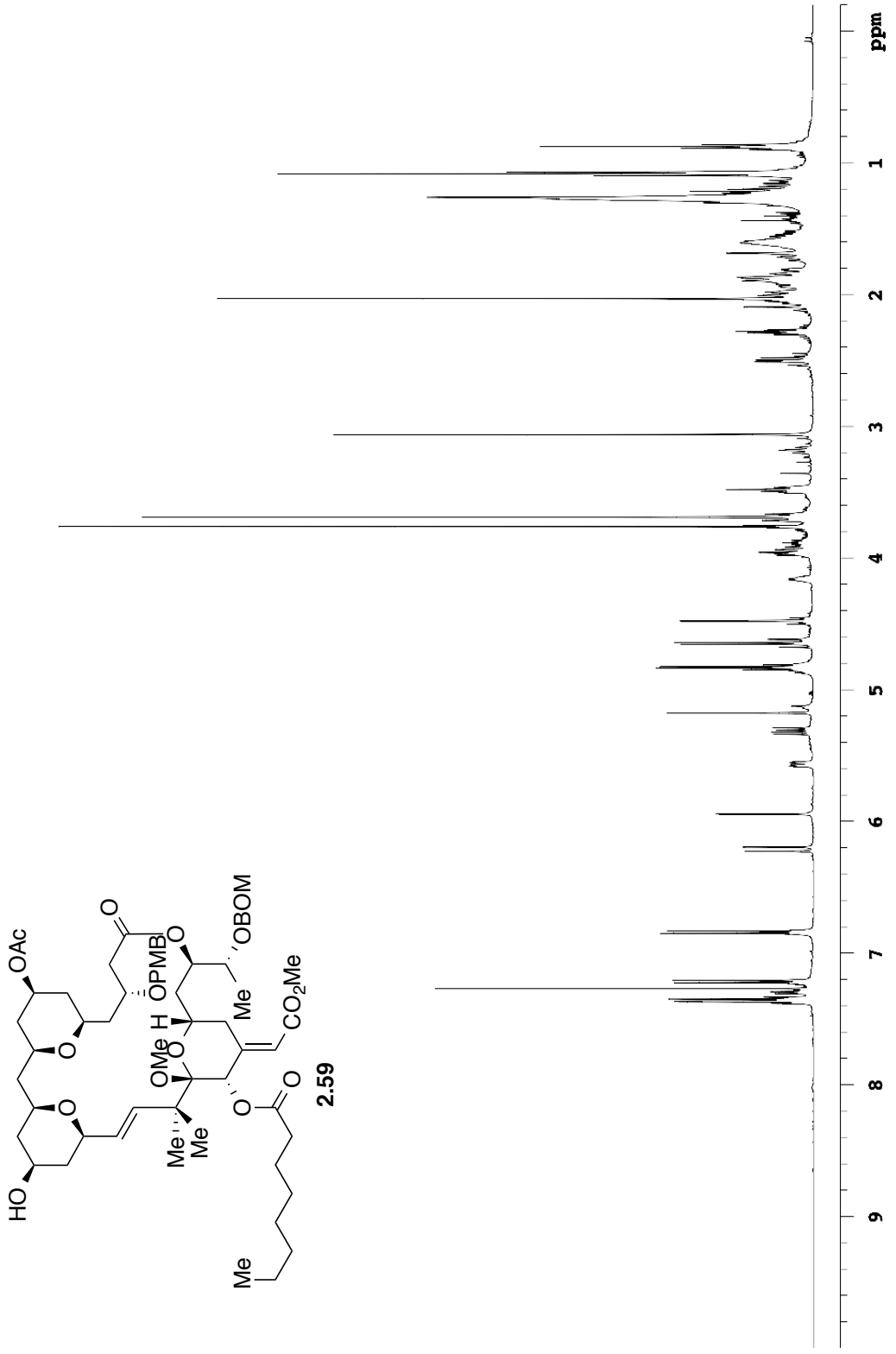


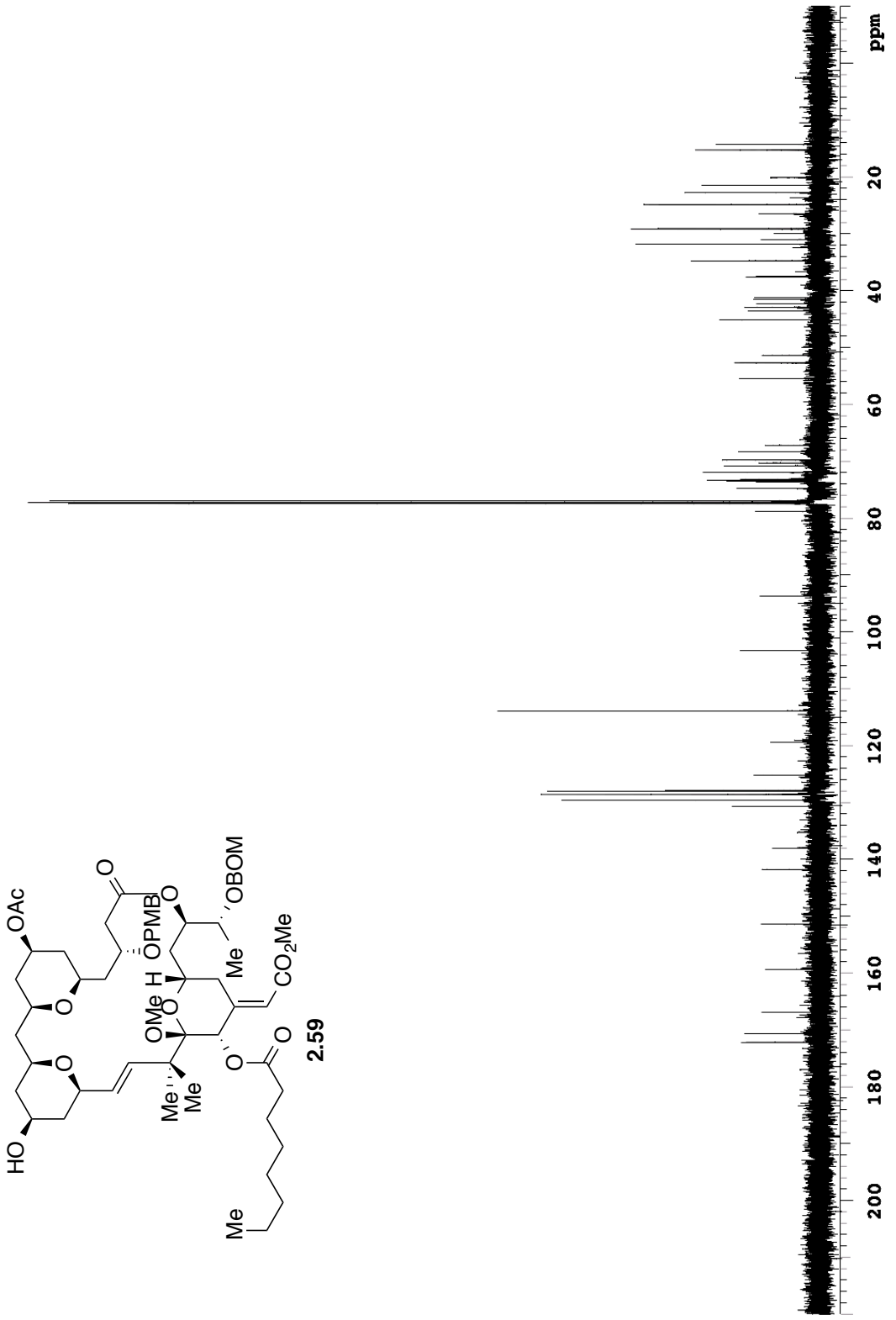
2.58

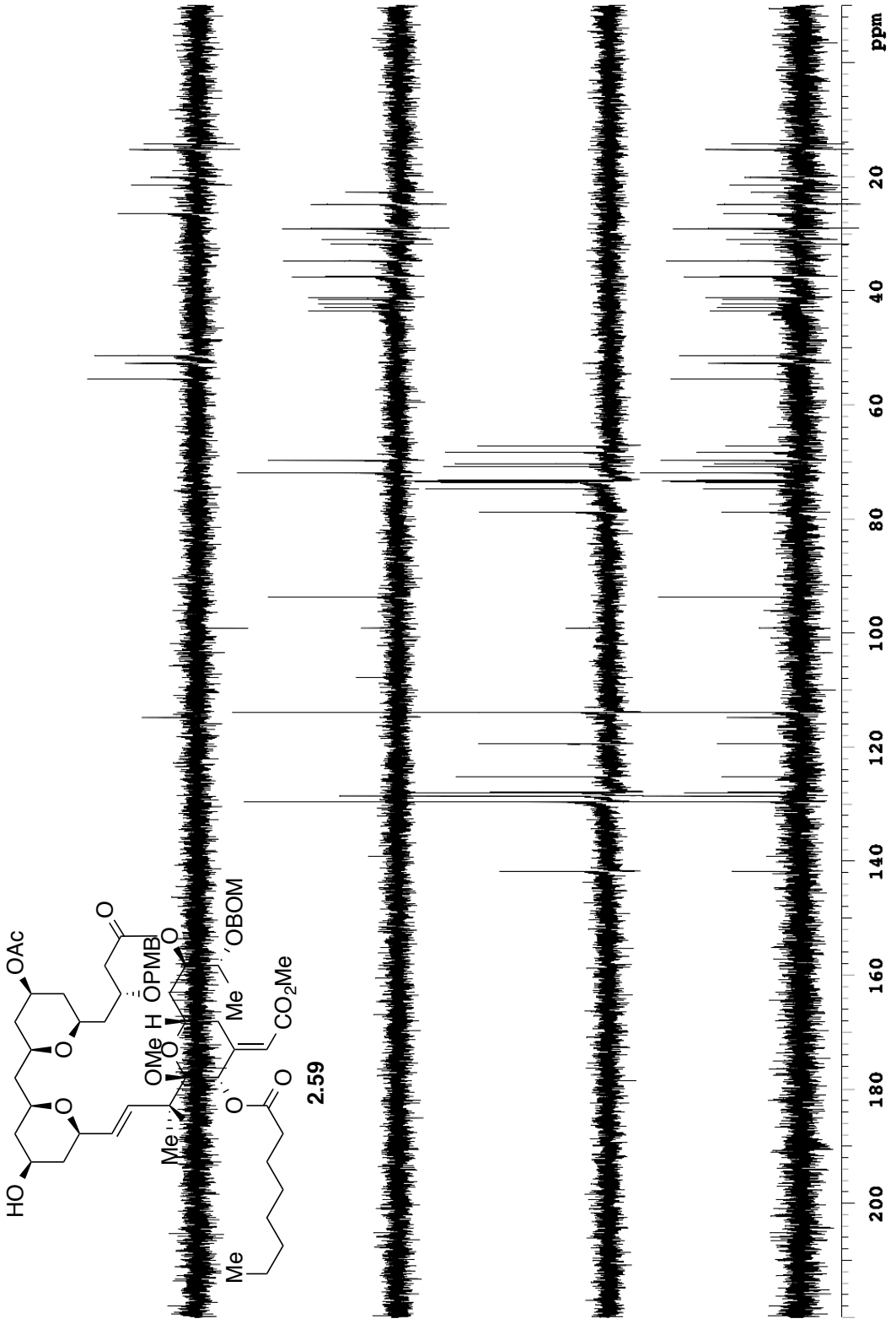

OAc
OPMB
OMe H
Me
Me
OBOM
Me
CO2Me
Me
ppm
200
180
160
140
120
100
80
60
40
20

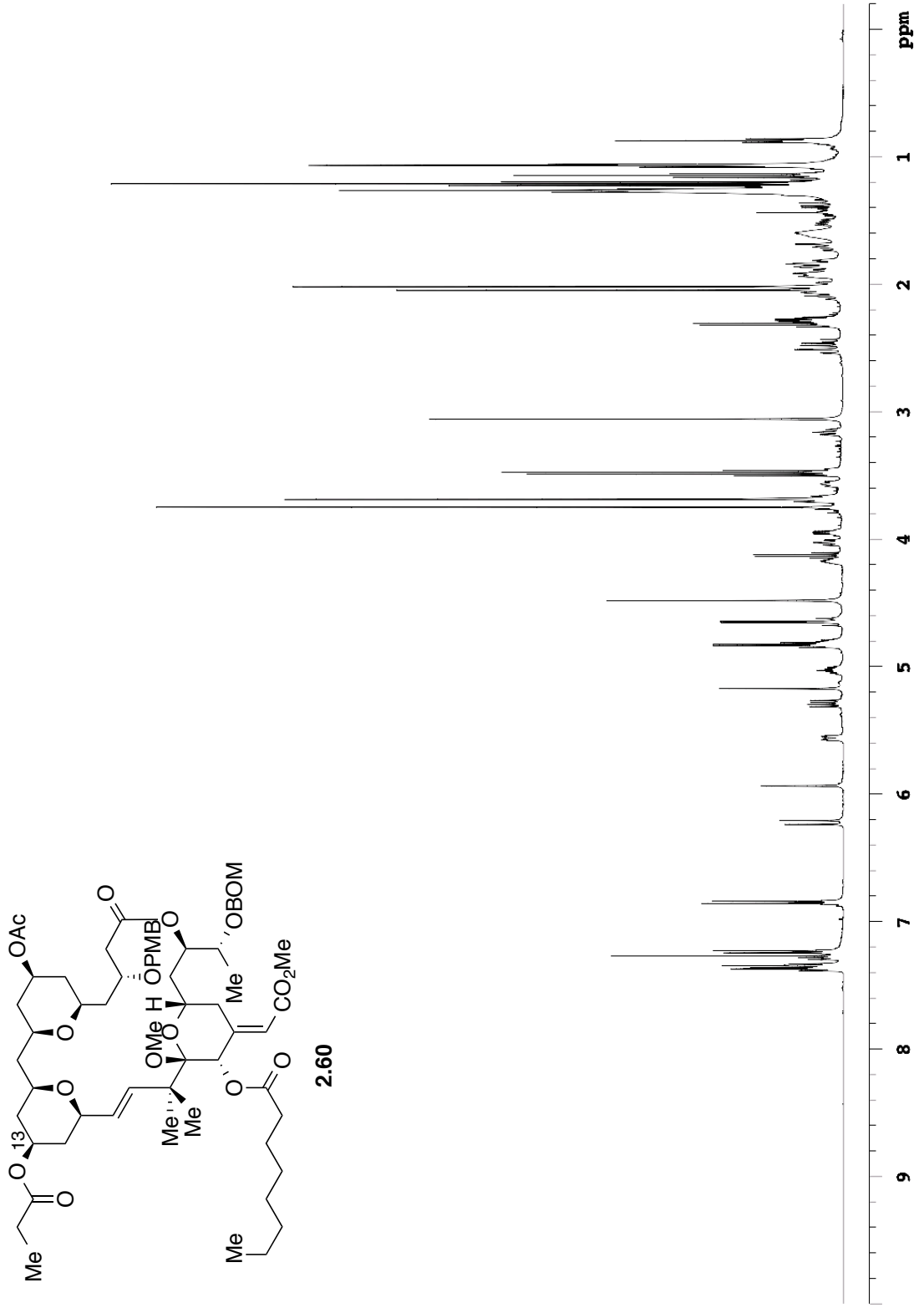
2.58


OAc
O
O
O
OMe H OPMB
O
O
Me
Me
Me
OBOM
CO2Me
O
O
Me
2.58
200
180
160
140
120
100
80
60
40
20
0
ppm

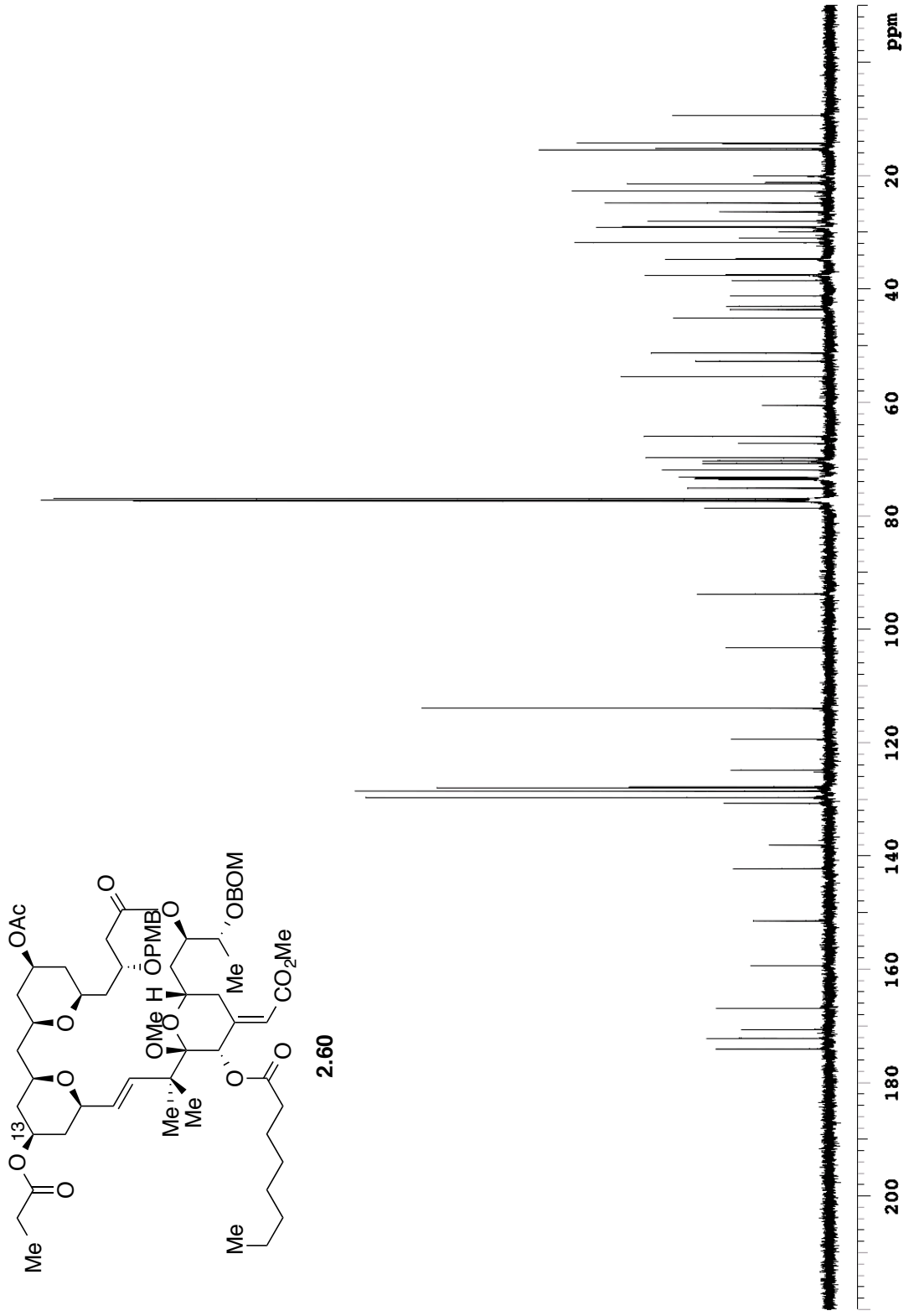

OAc
HO
OMe
H
OPMB
OBOM
Me
Me
Me
CO2Me
Me
2.59
ppm
1
2
3
4
5
6
7
8
9


OAc
HO
OMe H OPMB
Me
Me
Me
OBOM
CO2Me
Me
2.59
ppm
200
180
160
140
120
100
80
60
40
20

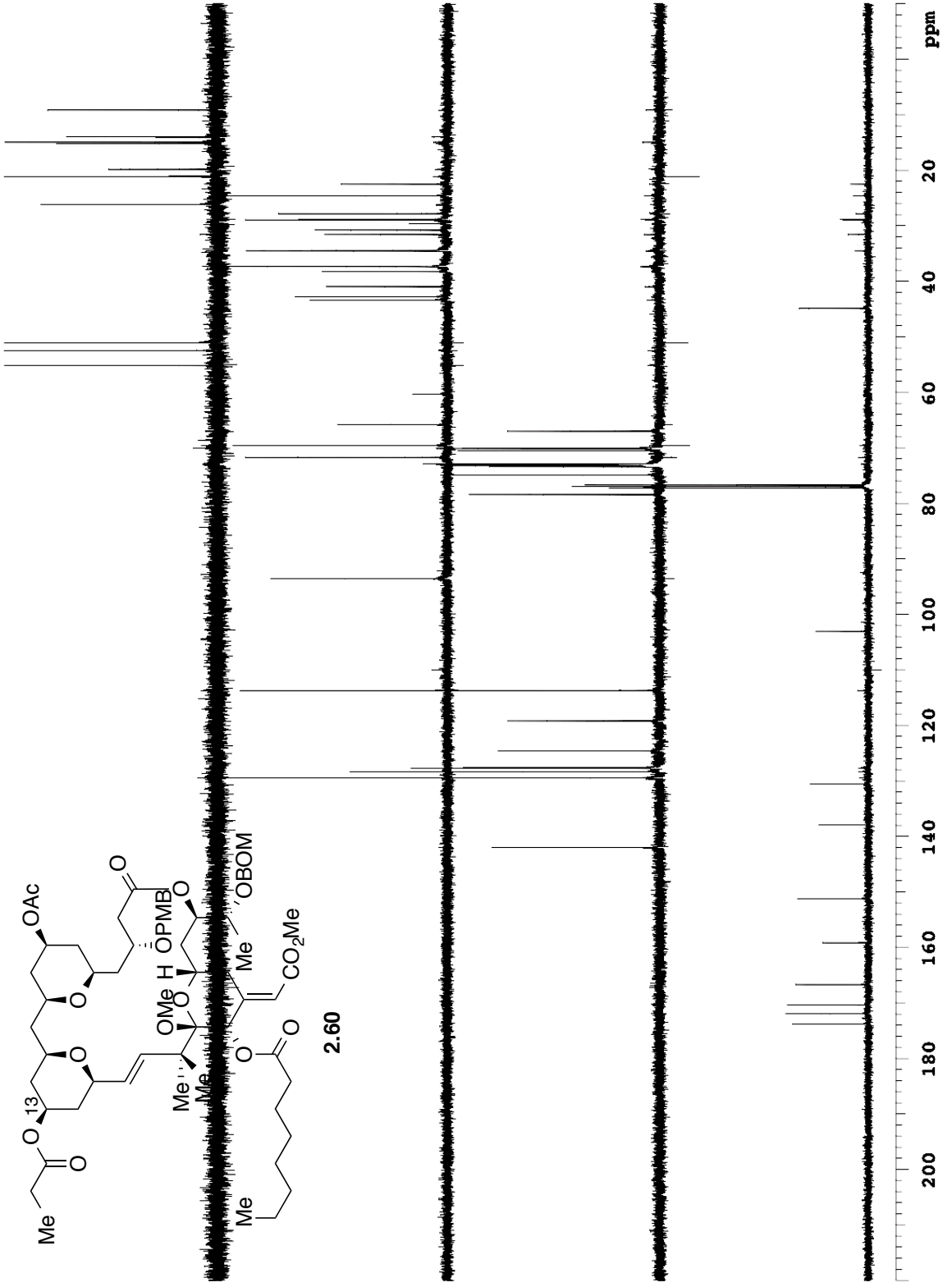

OAc
HO
OMe
H
OPMB
Me
OBOM
CO2Me
Me
2.59
ppm
200
180
160
140
120
100
80
60
40
20

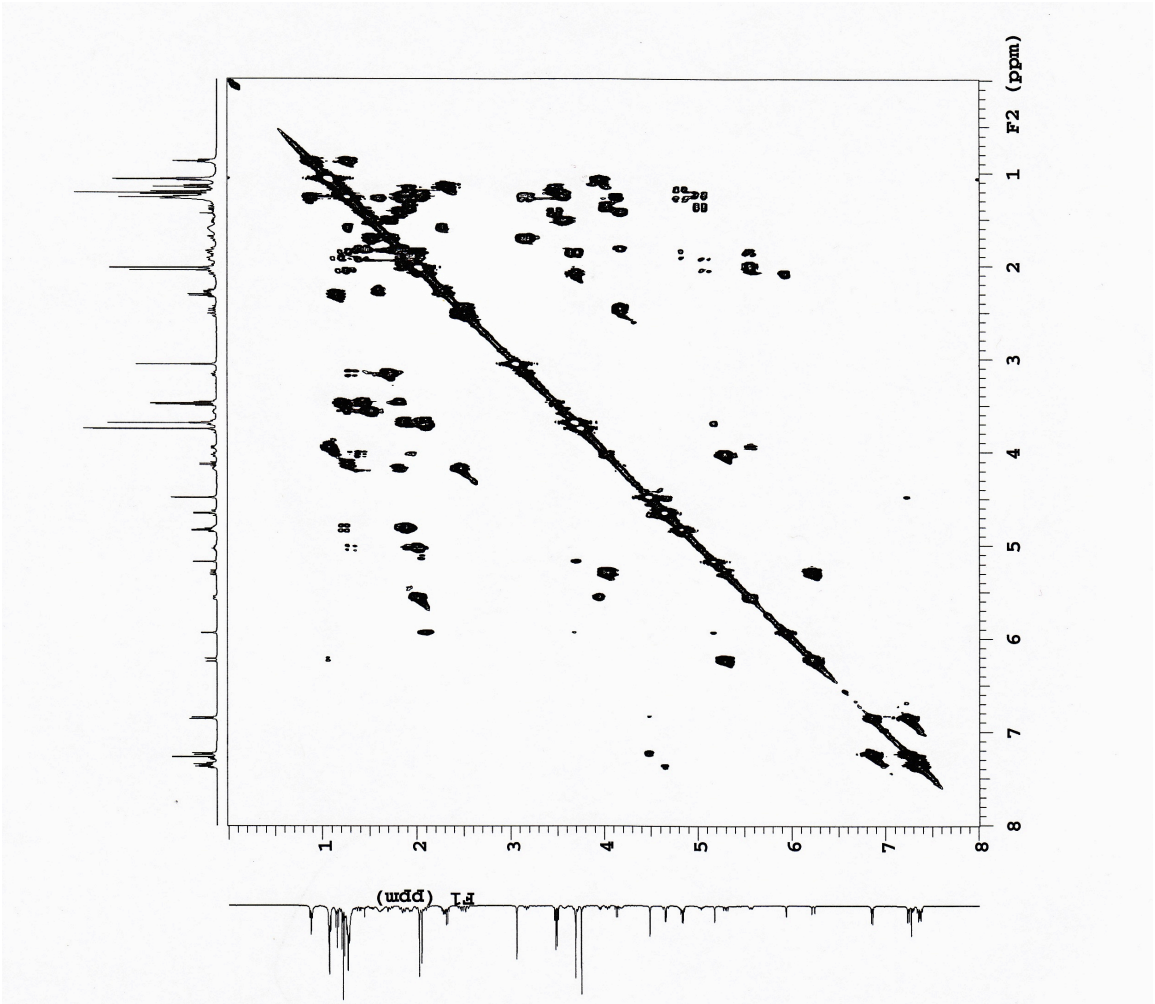


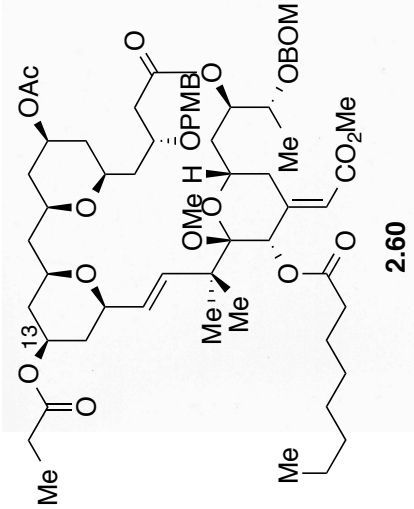
2.60


OAc
OPMB
OMe
H
OBOM
Me
Me
Me
Me
CO_2Me
13
ppm
20
40
60
80
100
120
140
160
180
200

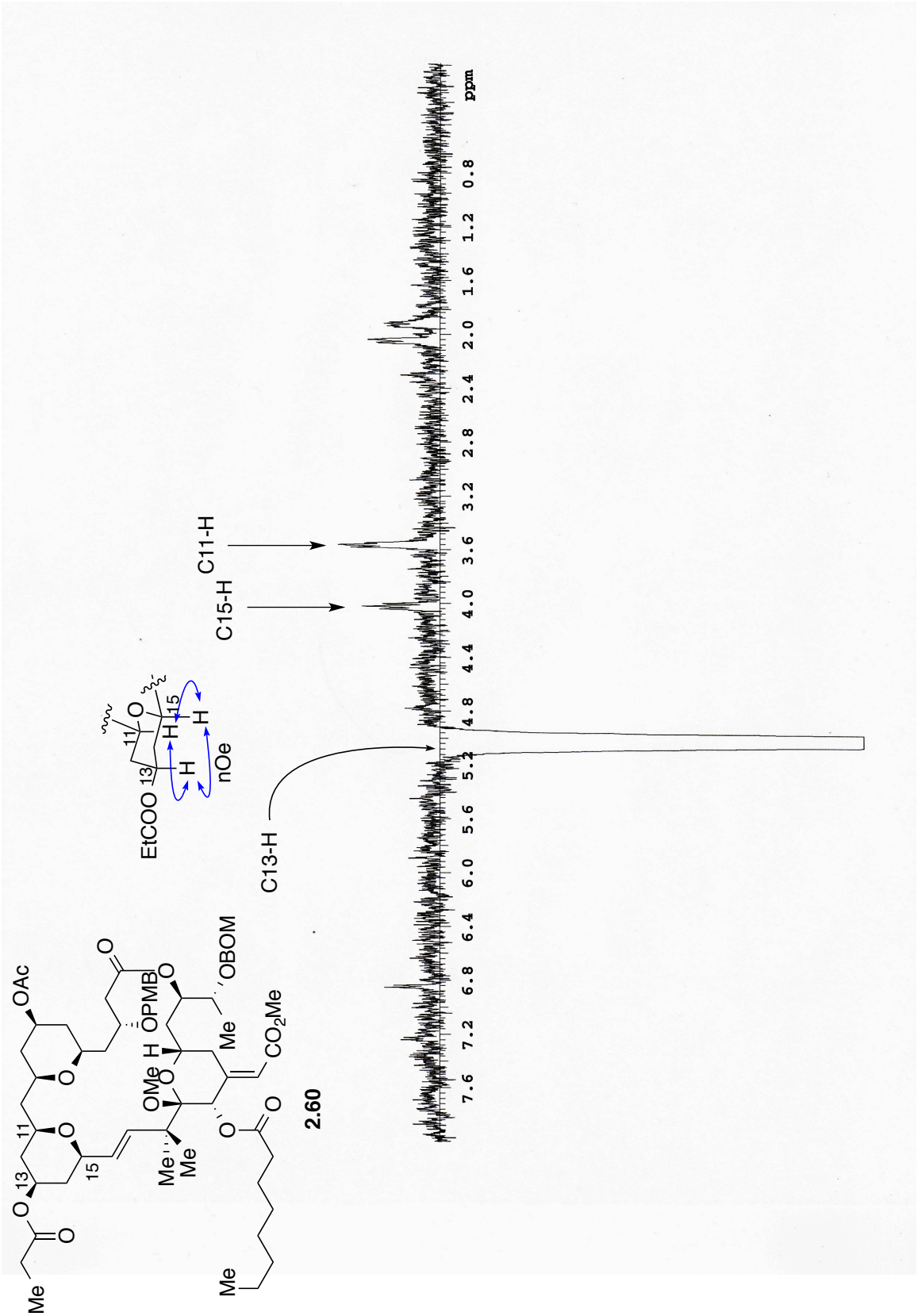
2.60

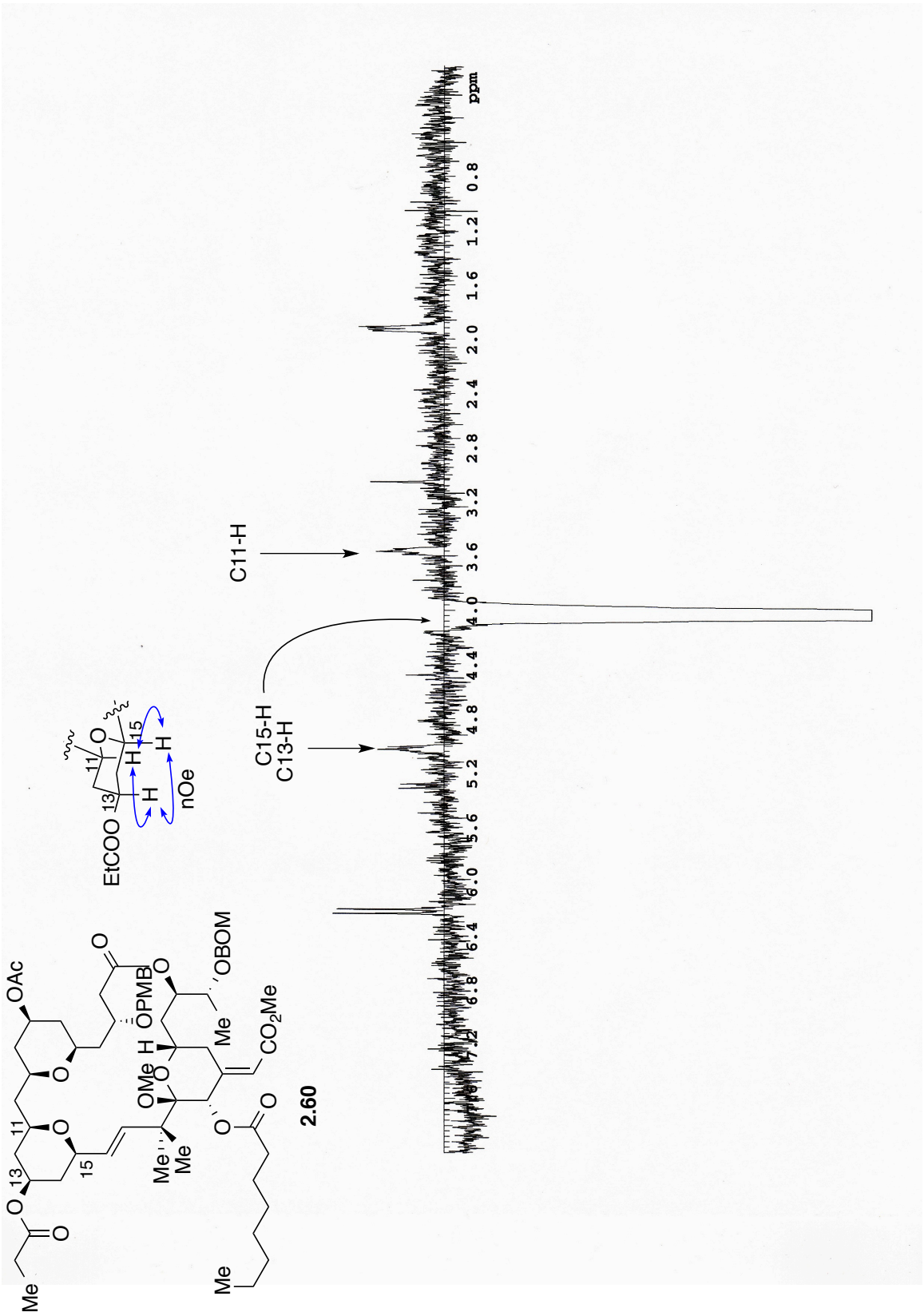

OAc
OMe H OPMB
OBOM
Me
CO2Me
Me
Me
Me
Me
O
13
2.60
ppm
20
40
60
80
100
120
140
160
180
200

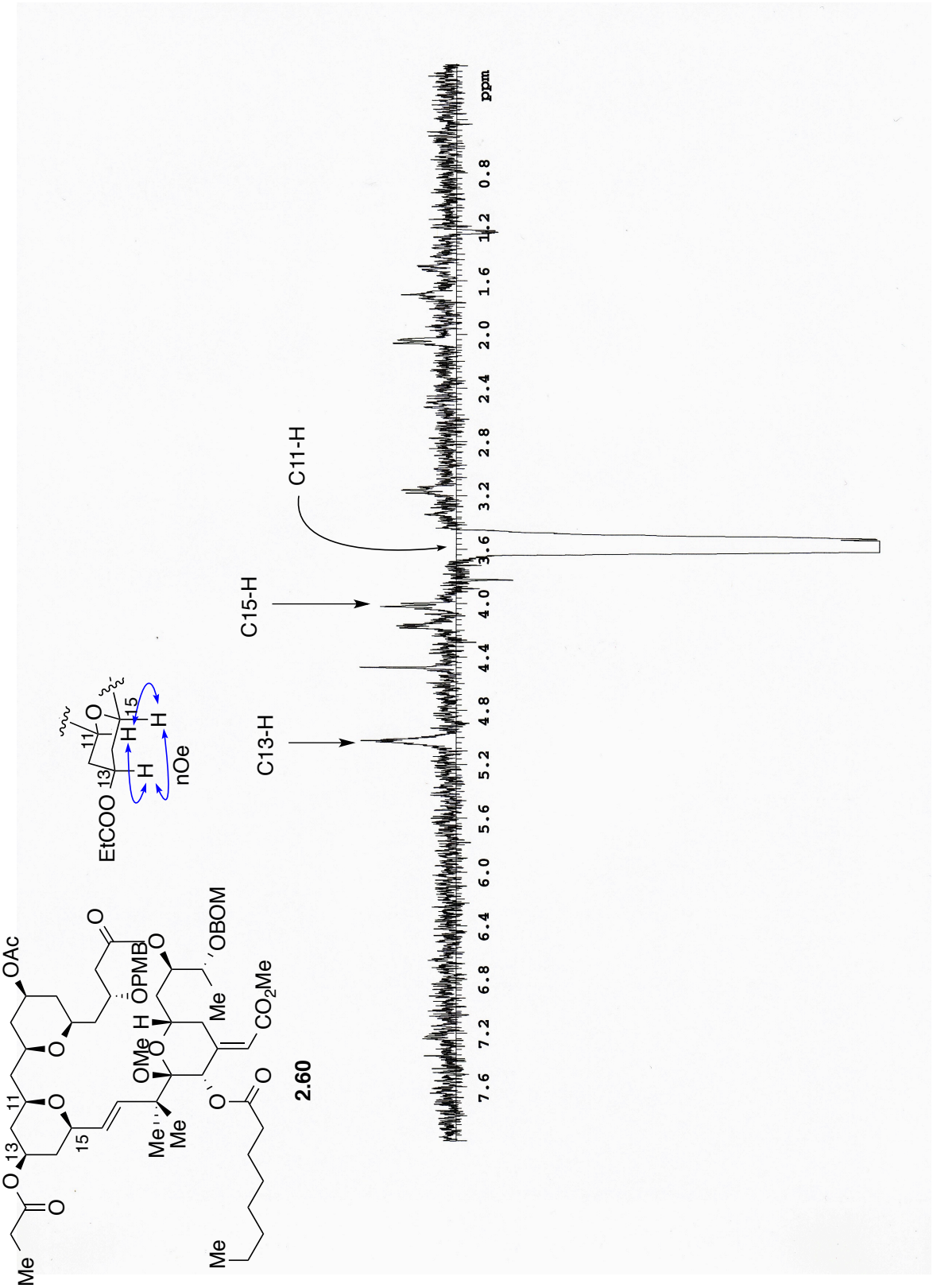


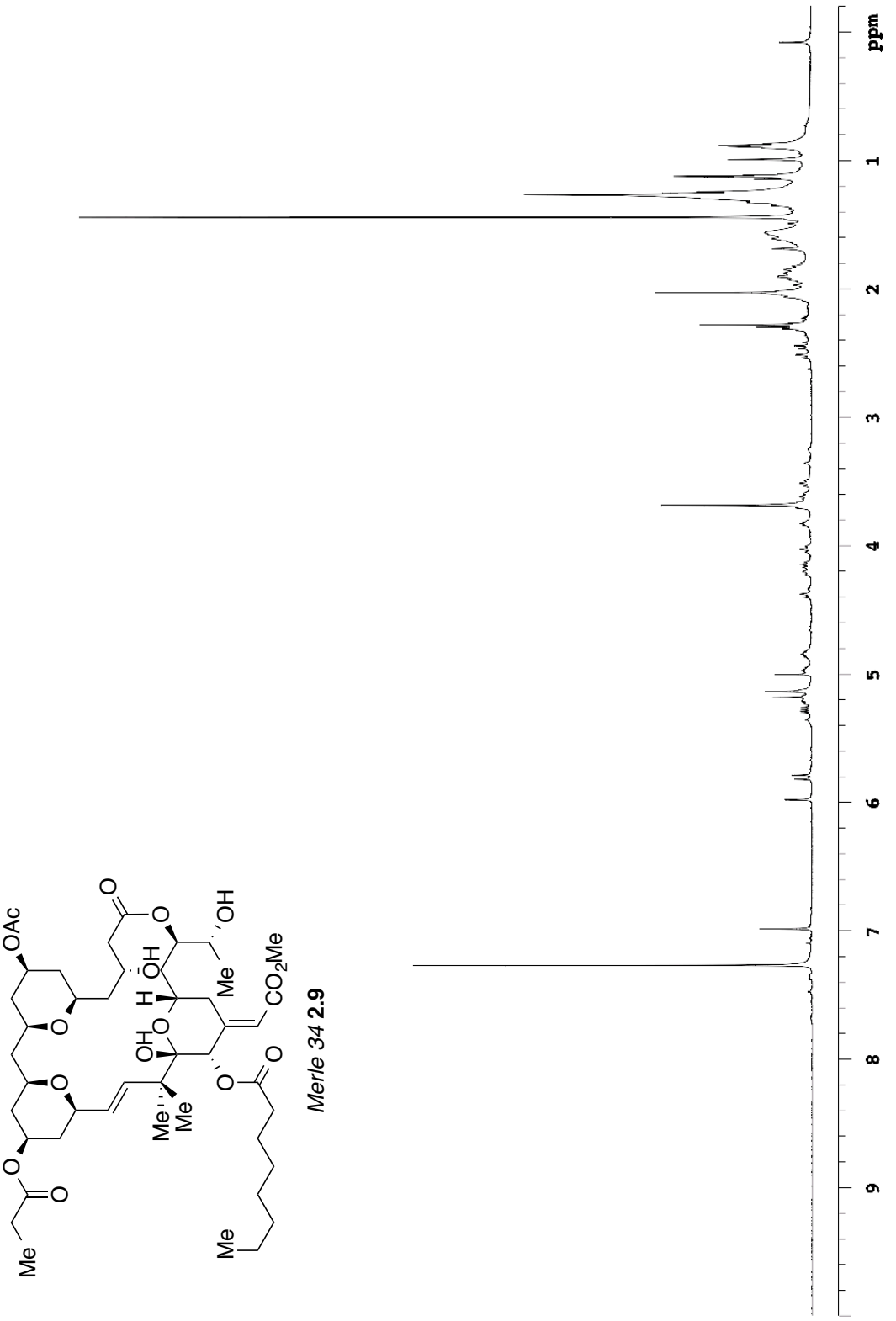


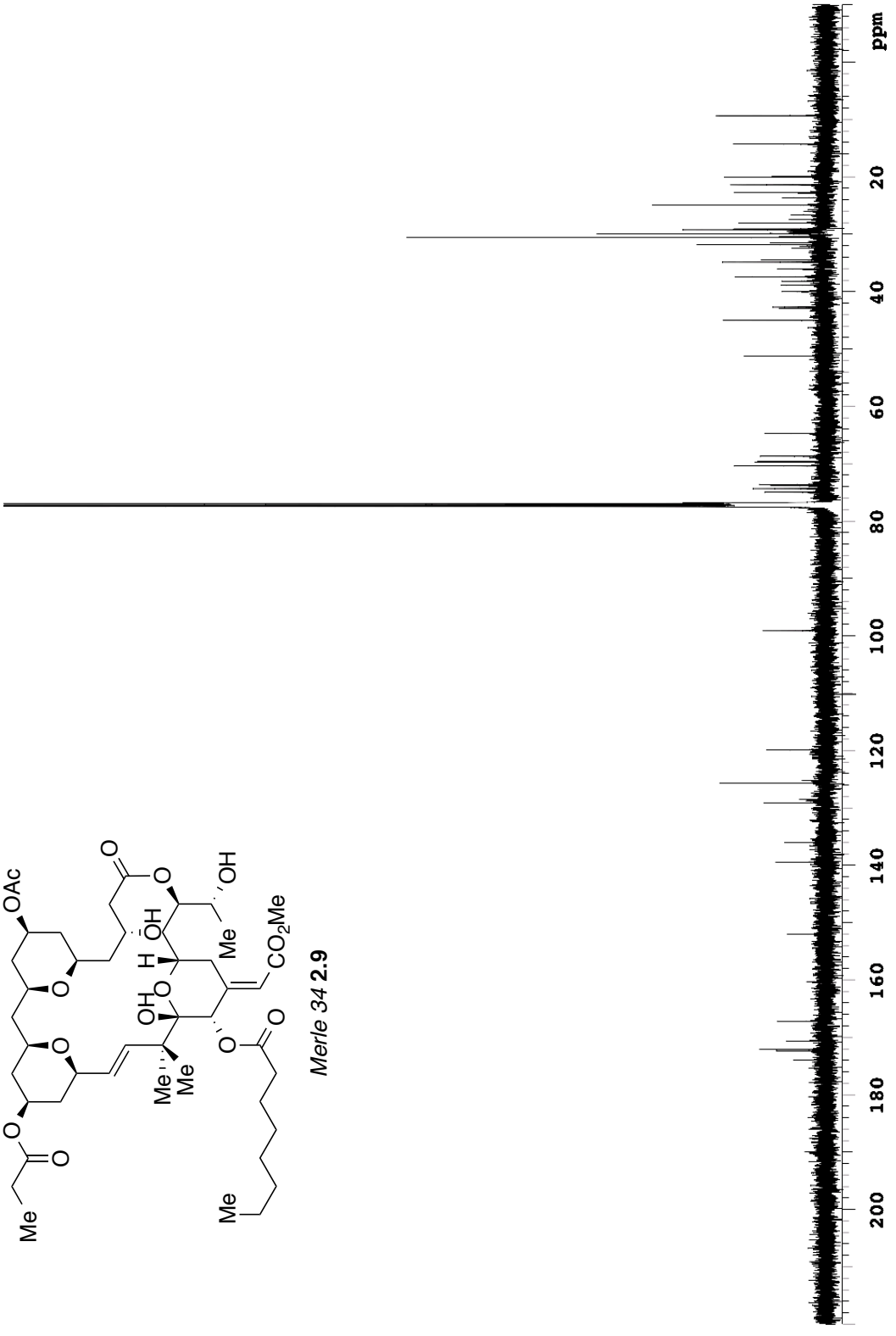
2.60


C11-H
C15-H
C13-H
nOe
EtCOO
OAc
OPMB
OBOM
OMe
Me
CO2Me
2.60
ppm

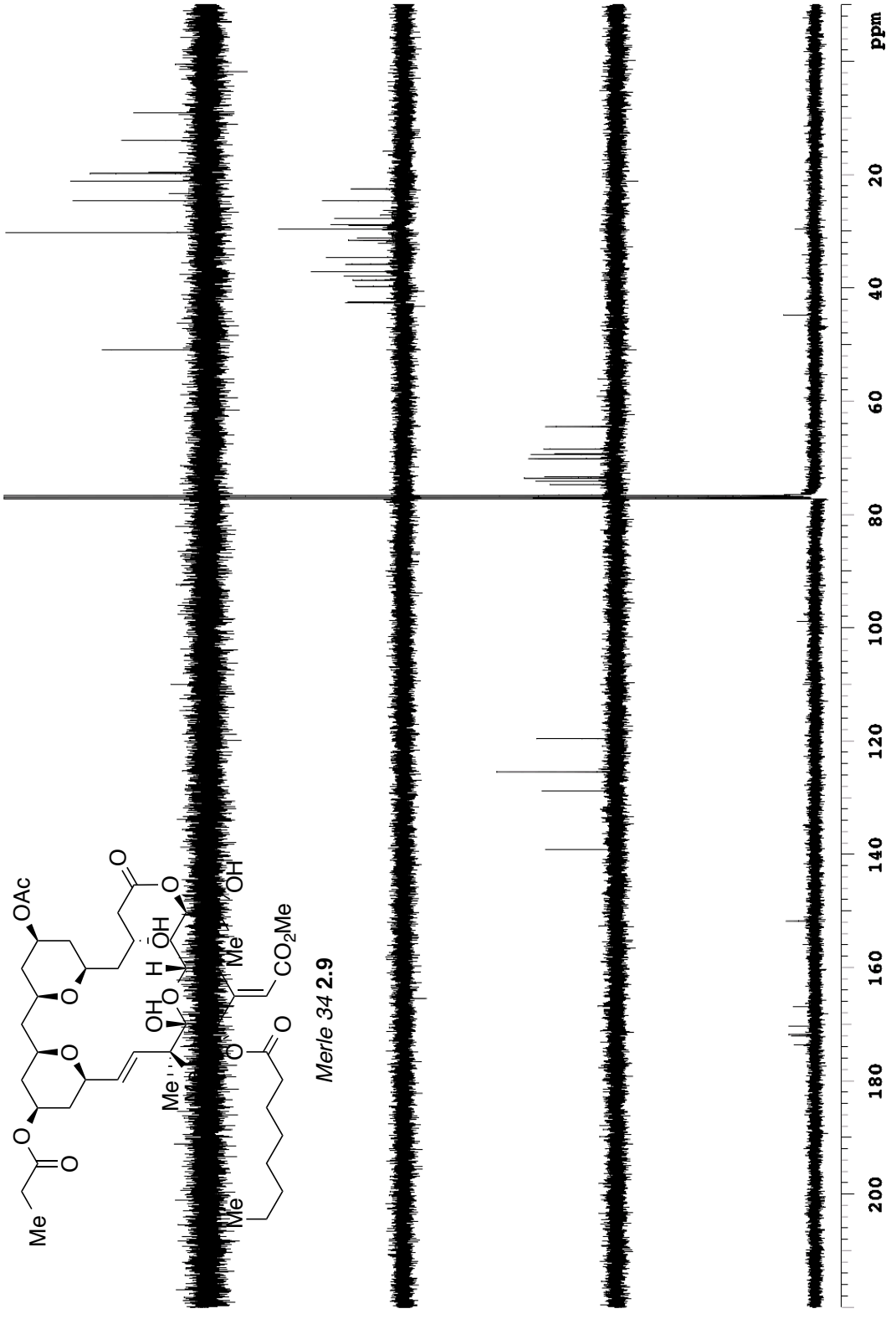

C11-H
C15-H
C13-H
ppm
EtCOO
nOe
OAc
OPMB
OBOM
OMe
CO2Me
Me
2.60


OAc
OPMB
OBOM
OMe
CO2Me
Me
EtCOO
nOe
C11-H
C15-H
C13-H
ppm
2.60

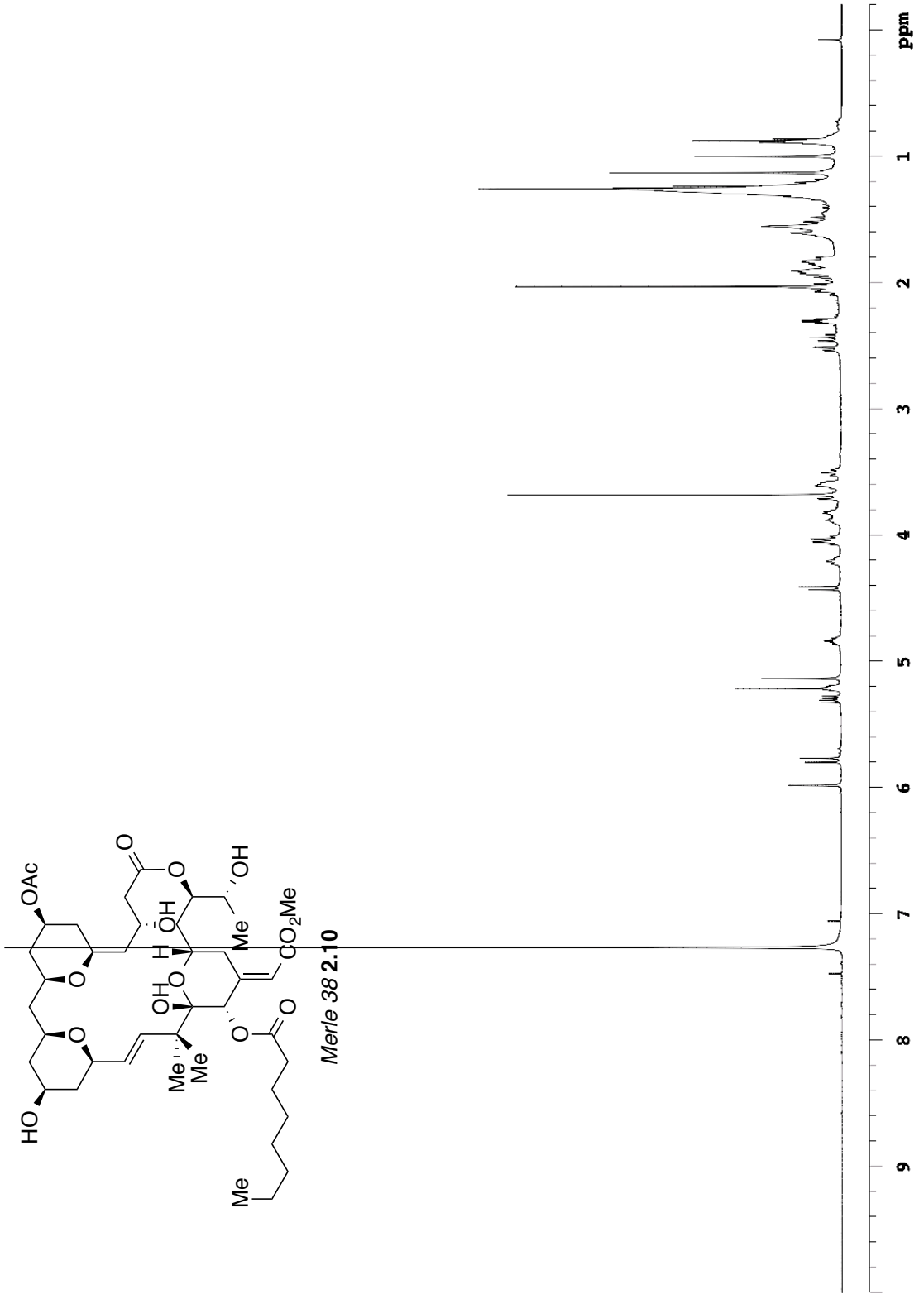

OAc
O
O
O


OAc
O
O
O
O
O
O
O
OH
H
OH
O
Me
Me
Me
OH
CO2Me
O
O
Me
Me
ppm
200
180
160
140
120
100
80
60
40
20

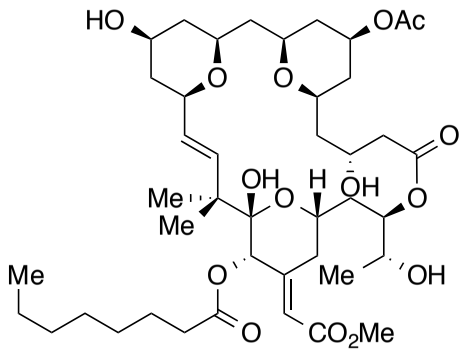
Merle 34 **2.9**


OAc
Me
O
O
O
O
O
O
OH
H
OH
O
Me
Me
OH
CO2Me
O
O
Me
200
180
160
140
120
100
80
60
40
20
ppm

Merle 34 **2.9**

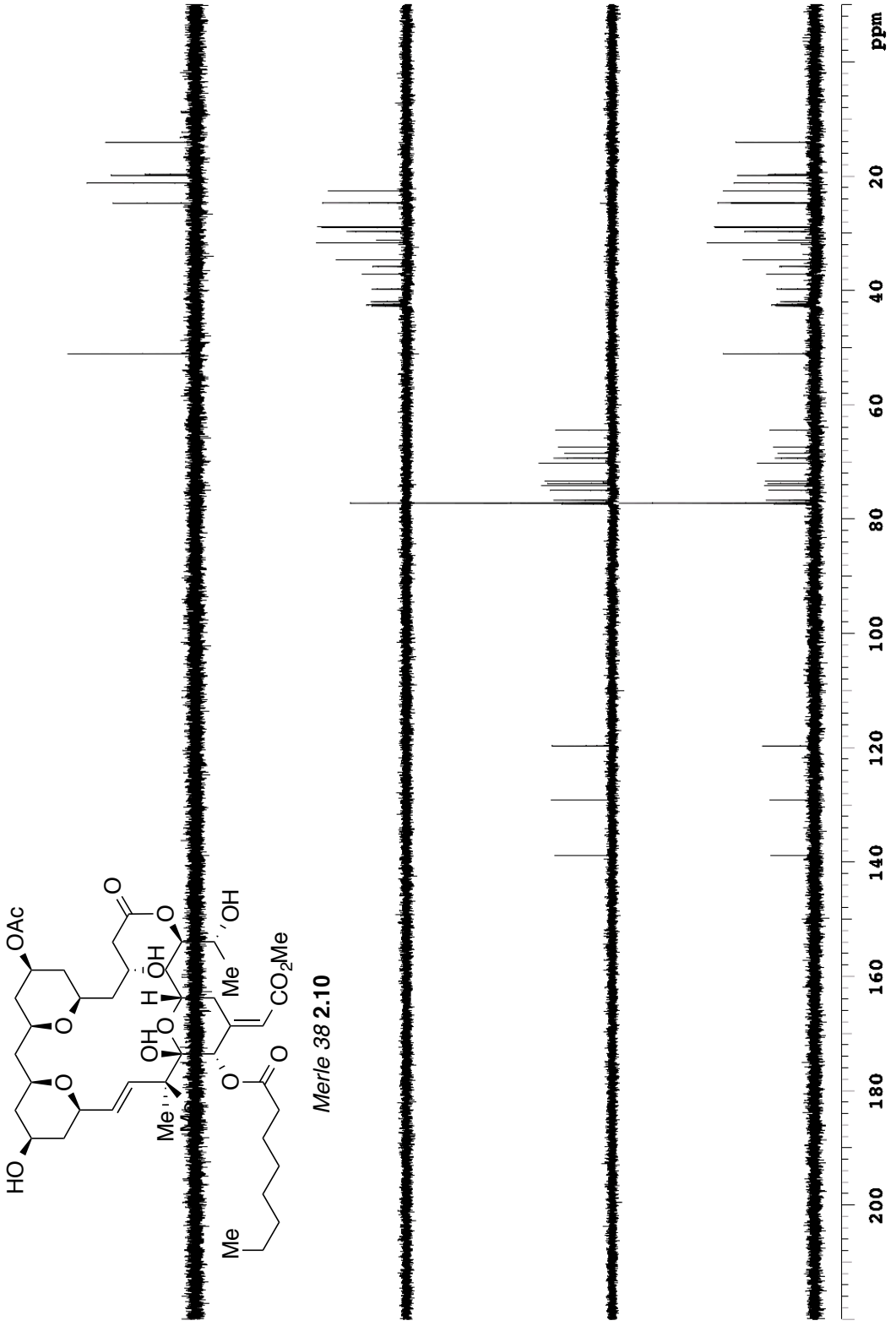


Merle 38 **2.10**


HO
OAc
O
O
O
OH
H
OH
O
O
Me
Me
Me
O
Me
OH
O
O
CO2Me

Merle 38 **2.10**

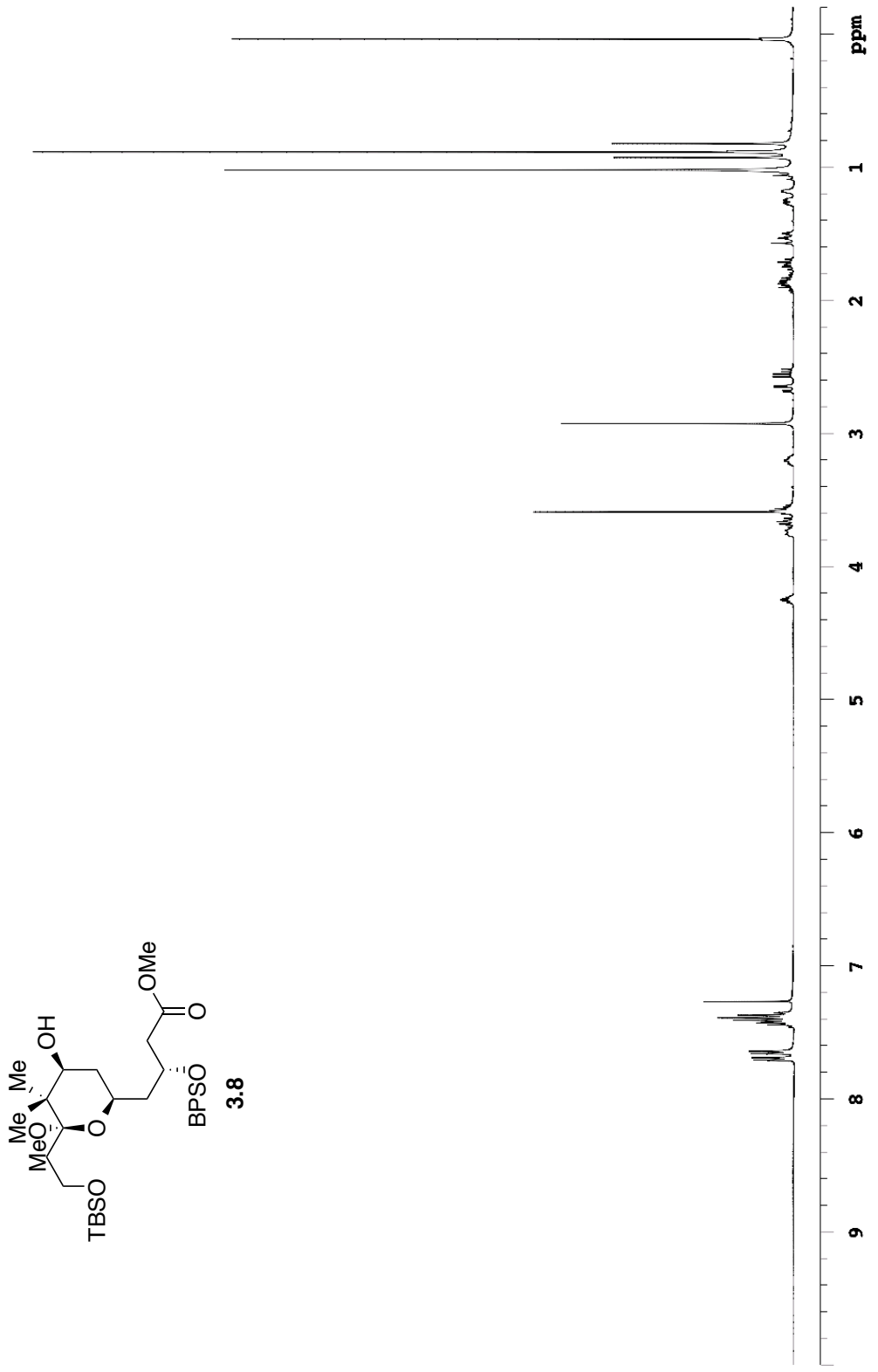
200
180
160
140
120
100
80
60
40
20
ppm

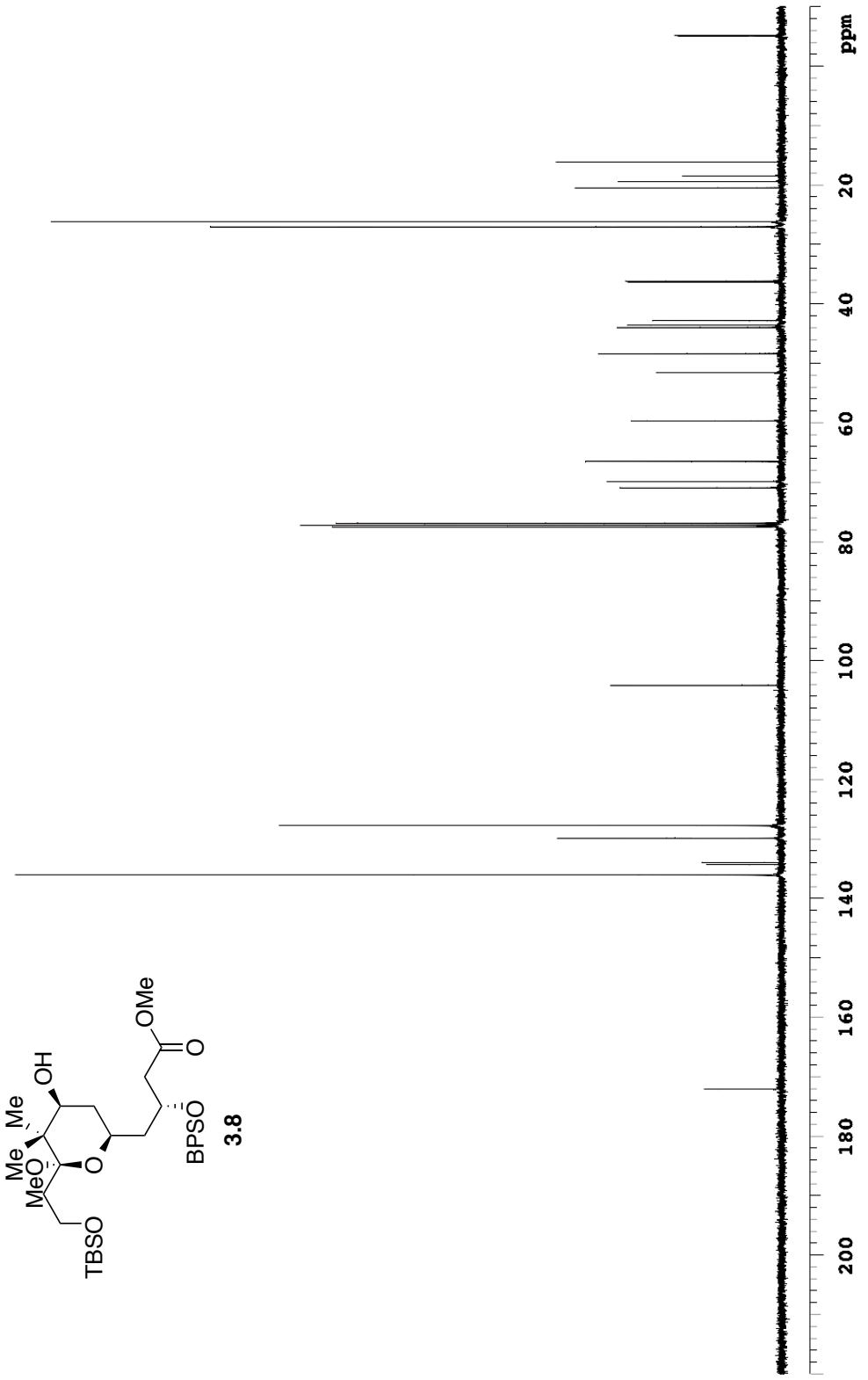

OAc
HO
OH
Me
Me
Me
OH
CO2Me
Me
ppm
200
180
160
140
120
100
80
60
40
20

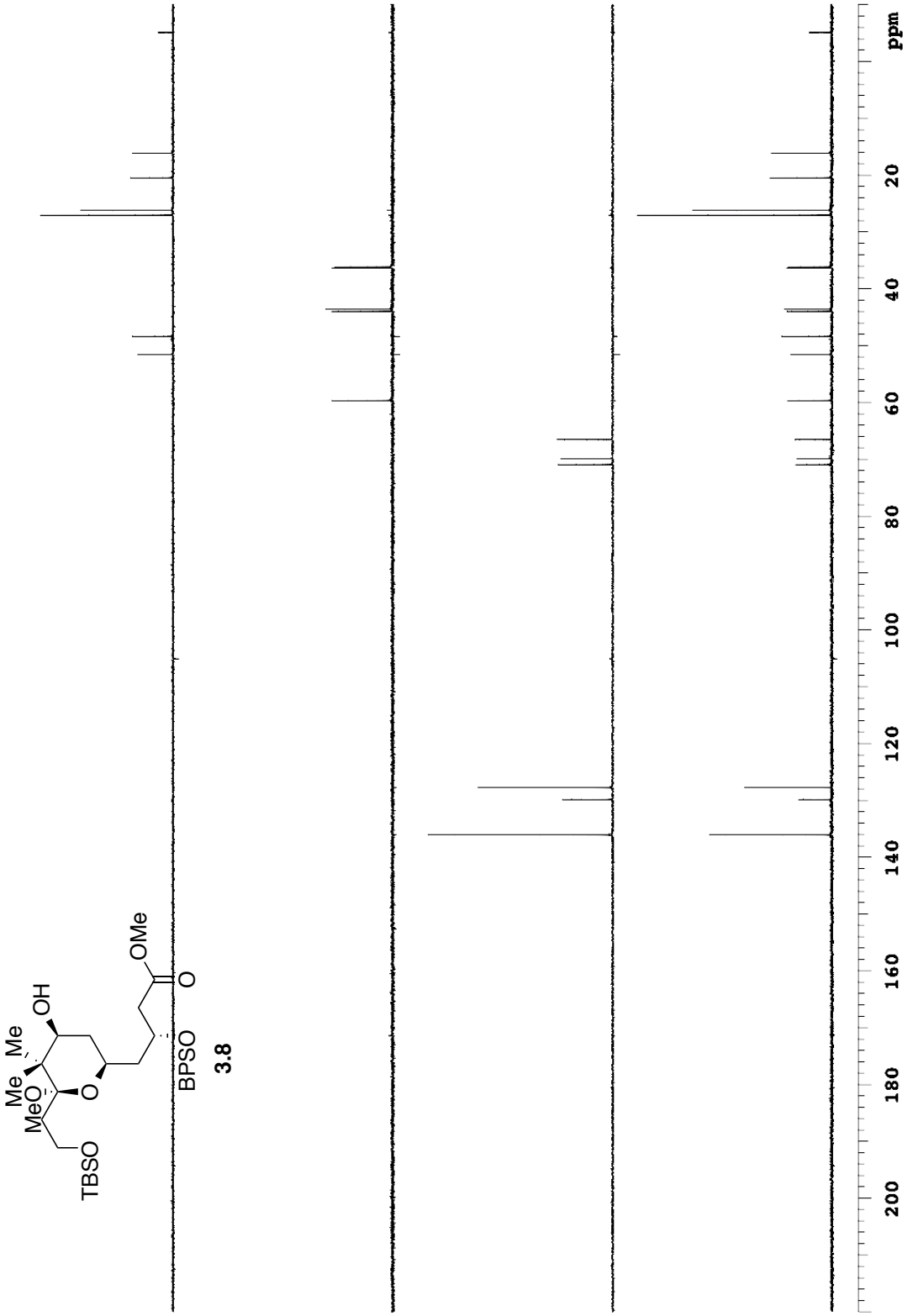
Merle 38 **2.10**

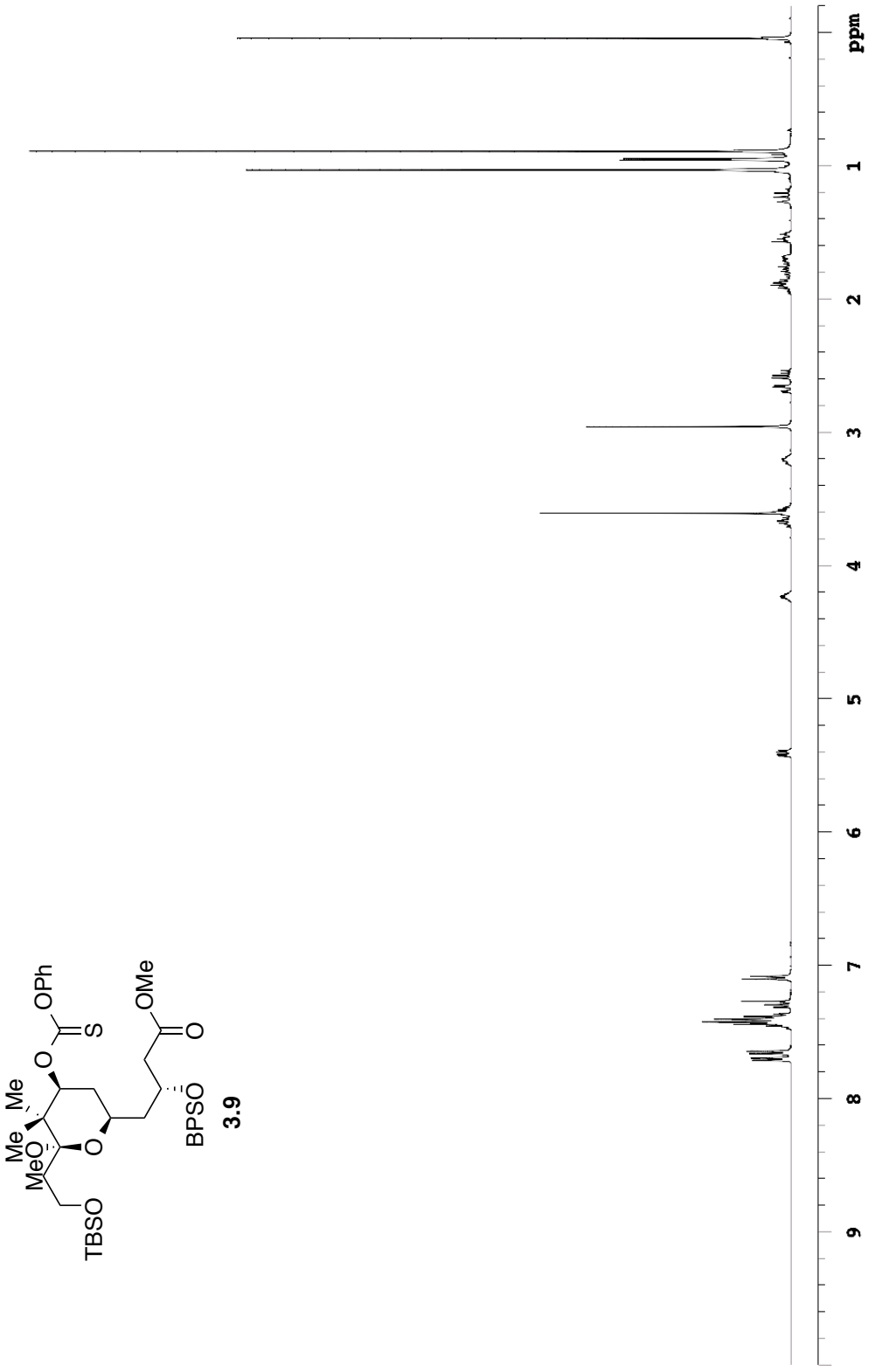
APPENDIX C

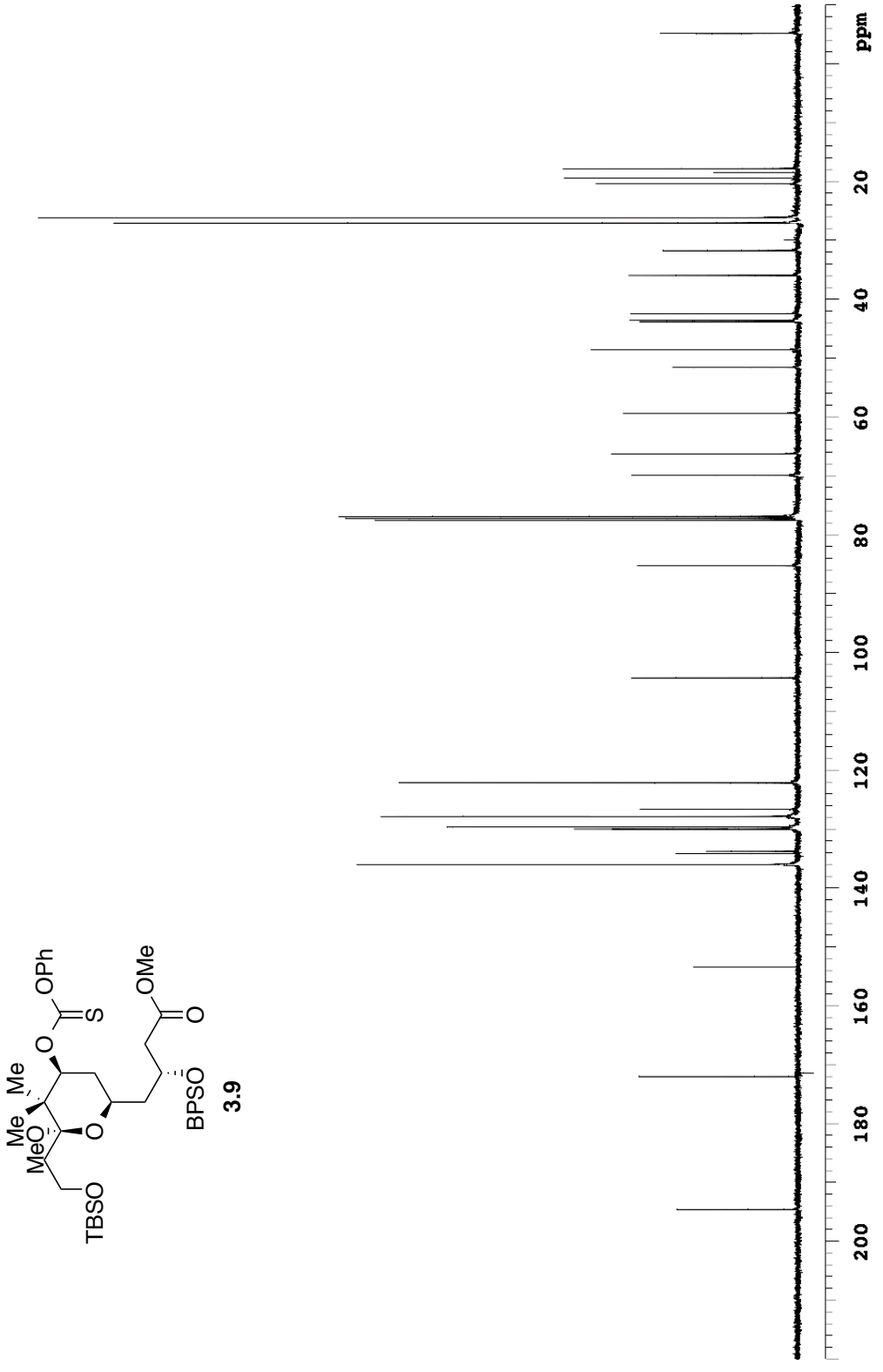
^{1}H AND ^{13}C NMR SPECTRA FOR CHAPTER 3

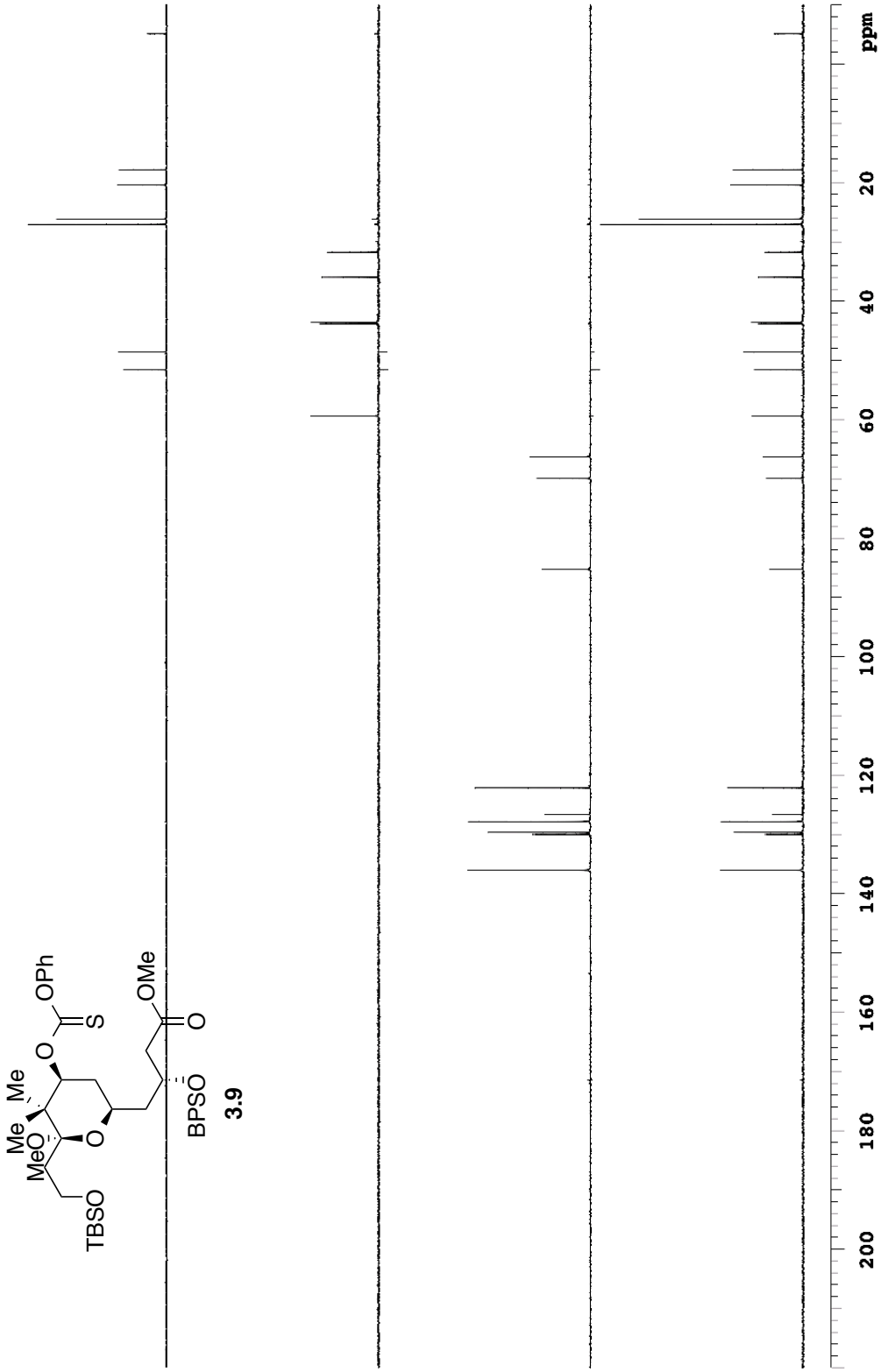

OMe
O
OH
Me
Me
MeO
O
BPSO
TBSO
3.8
ppm
1
2
3
4
5
6
7
8
9

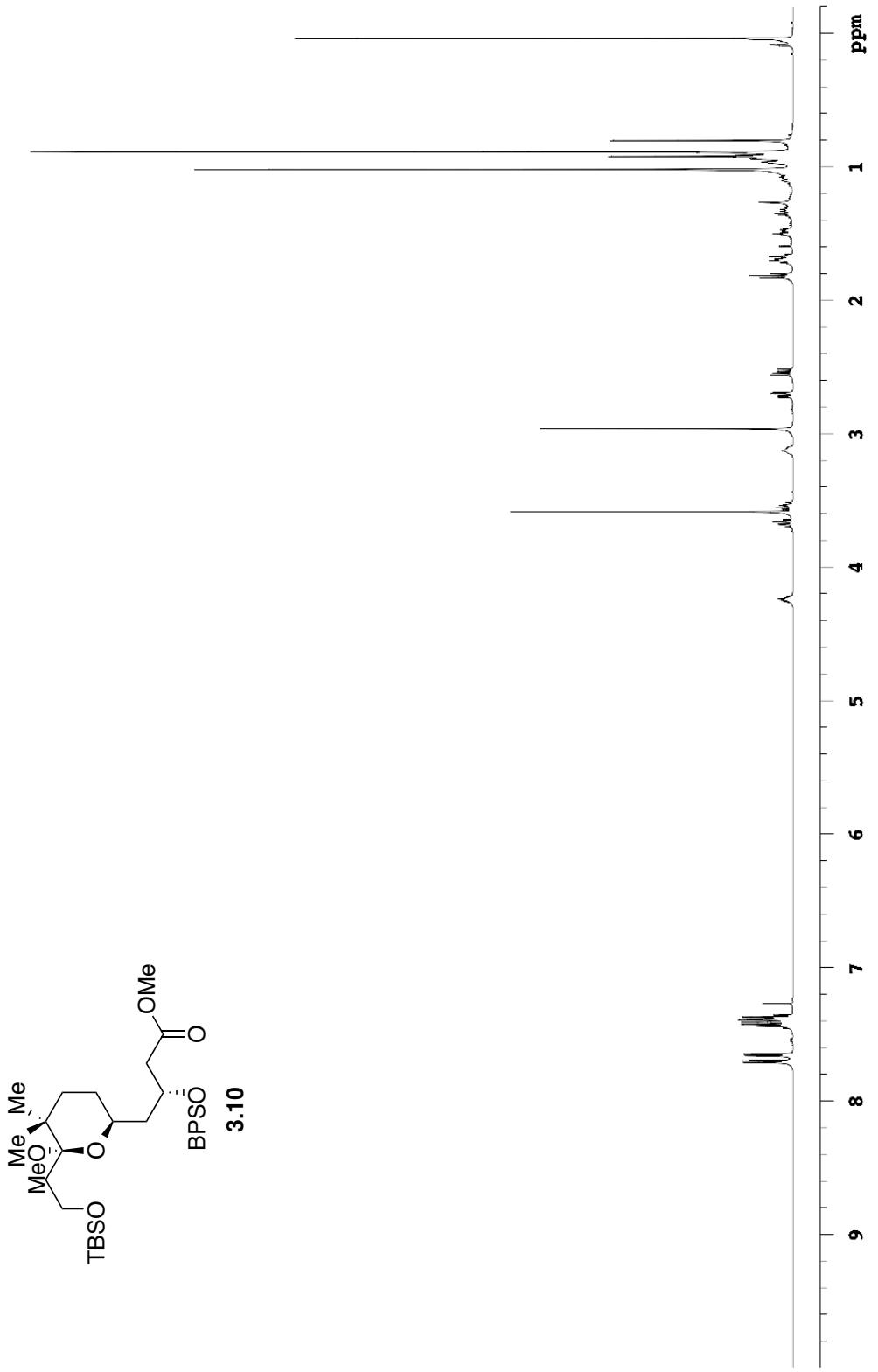

OMe
O
OH
Me
Me
MeO
O
BPSO
TBSO
3.8
ppm
200
180
160
140
120
100
80
60
40
20

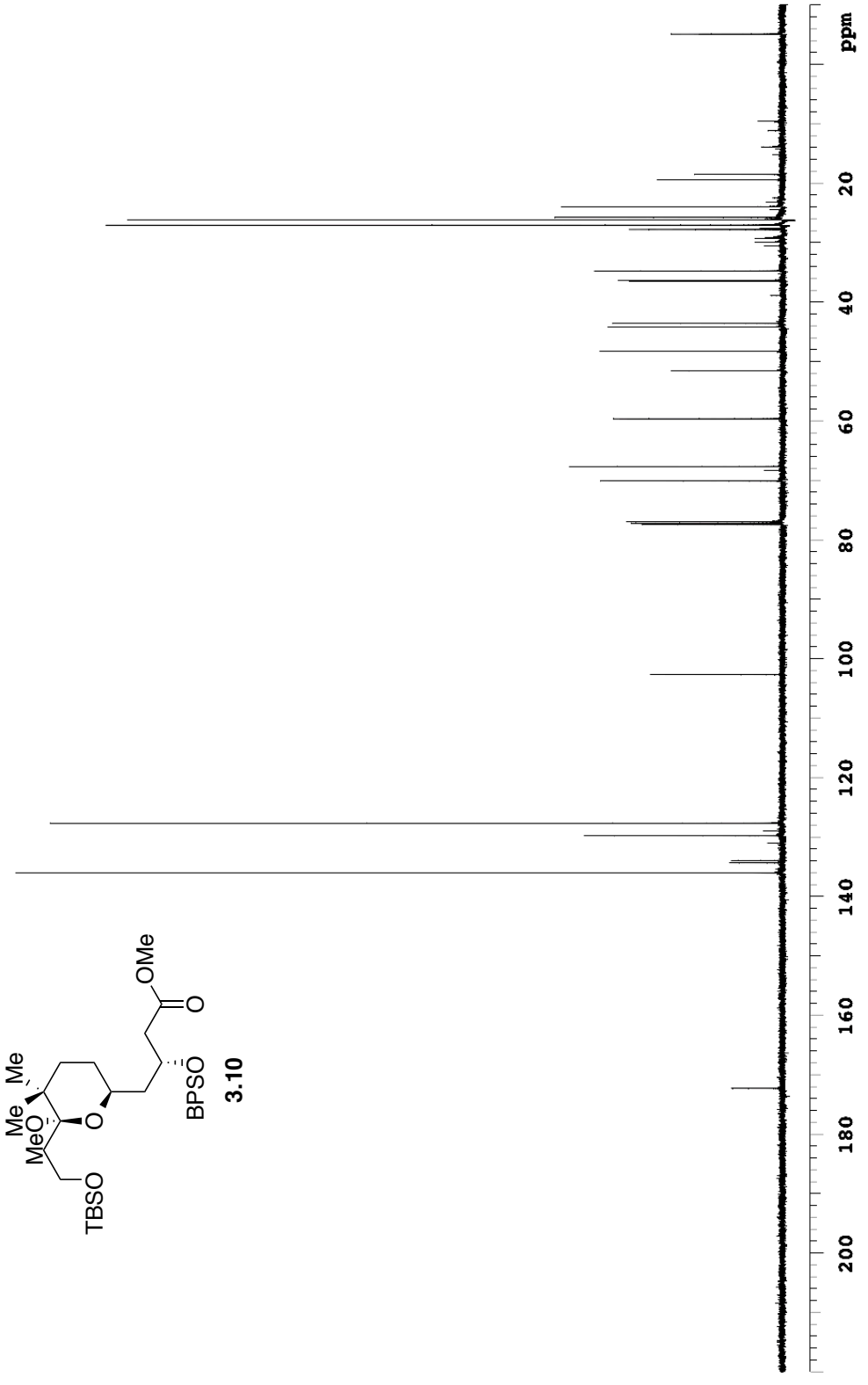

OMe
O
OH
Me
Me
MeO
O
TBSO
BPSO
3.8
ppm
20
40
60
80
100
120
140
160
180
200

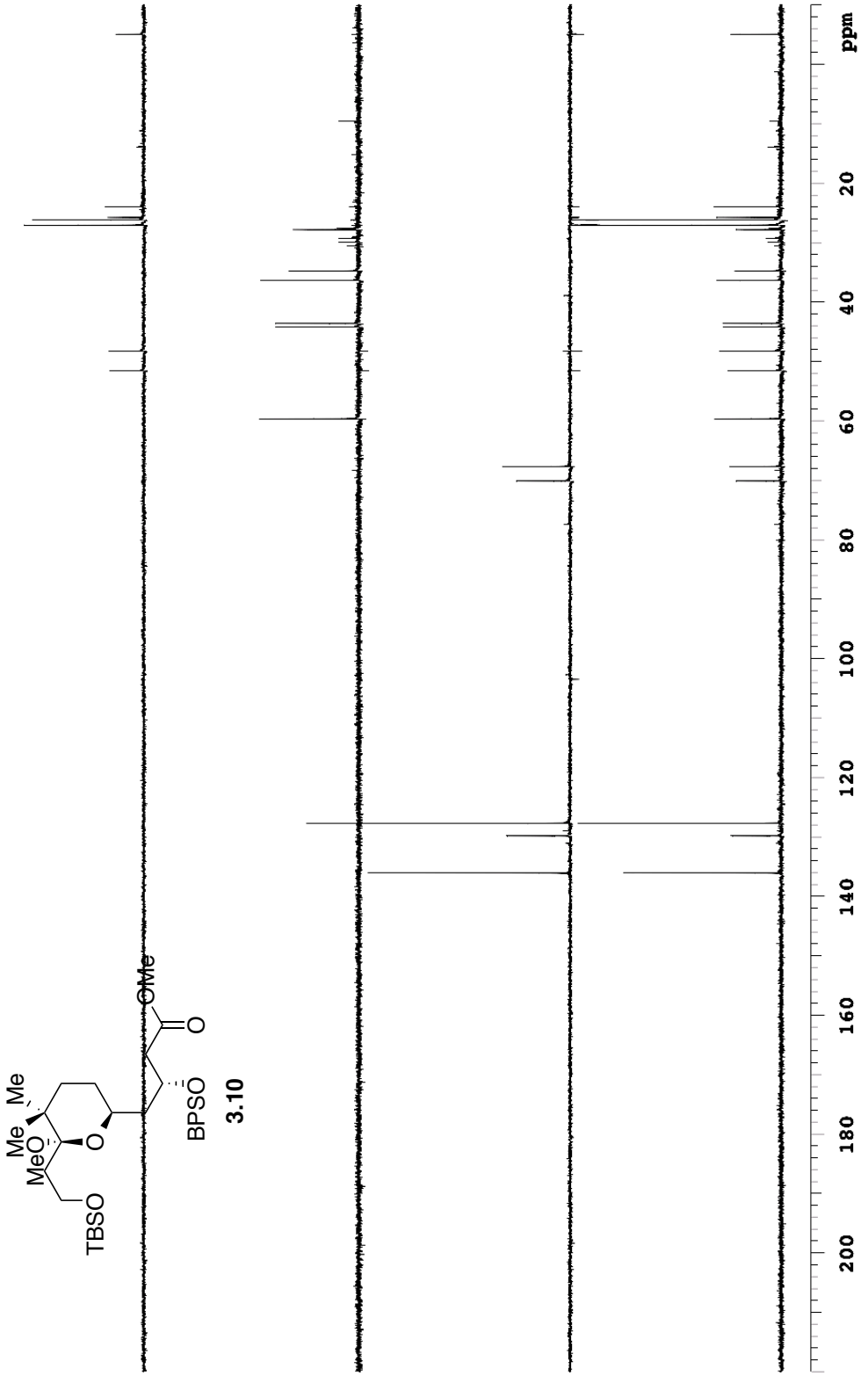

OPh
S
O
OMe
O
Me
Me
MeO
O
BPSO
TBSO
3.9
ppm
1
2
3
4
5
6
7
8
9

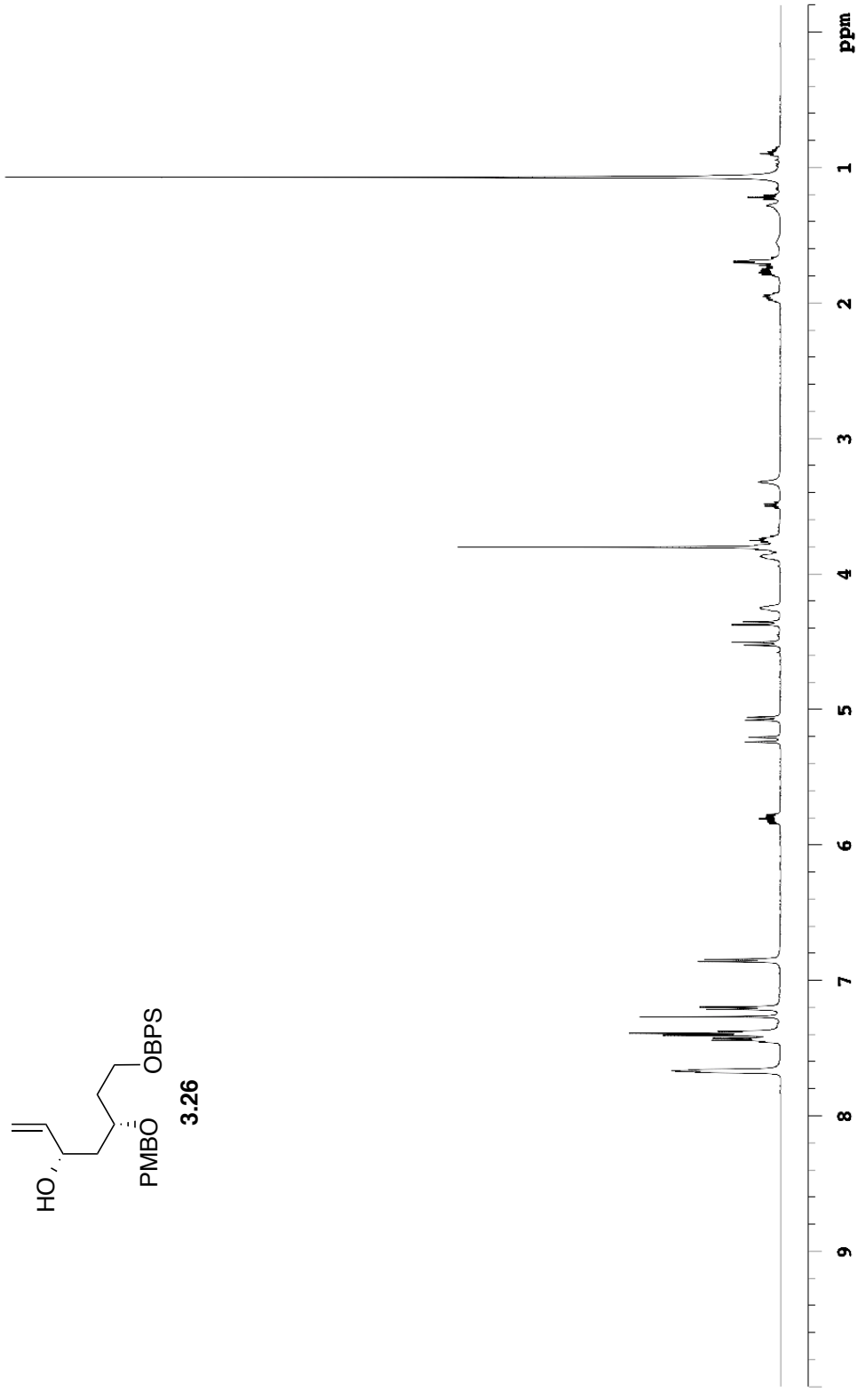

OPh
S
O
OMe
O
Me
Me
MeO
O
BPSO
TBSO
3.9
ppm
20
40
60
80
100
120
140
160
180
200

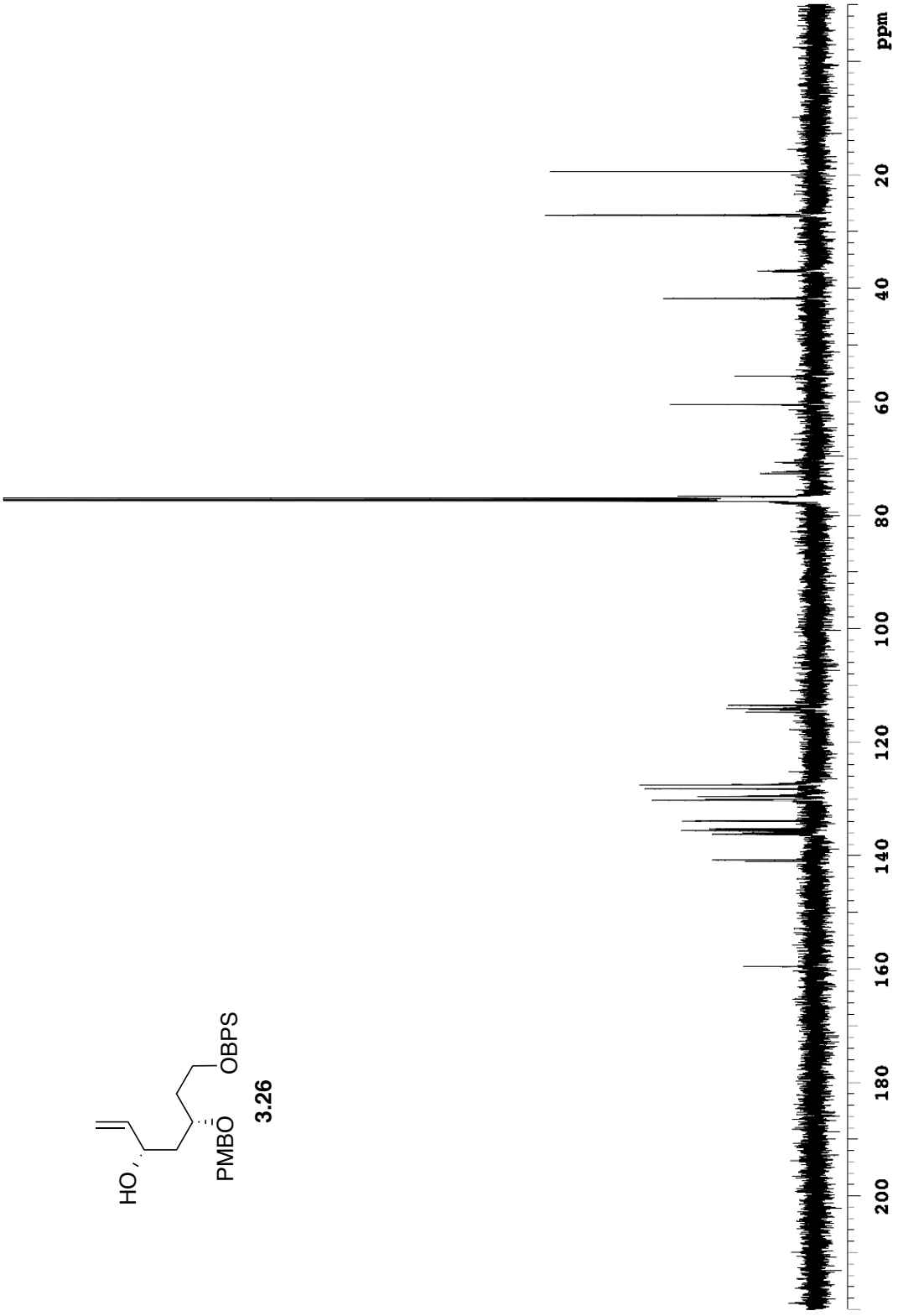

OPh
S
O
OMe
O
Me
Me
MeO
O
BPSO
TBSO
3.9
ppm
200
180
160
140
120
100
80
60
40
20

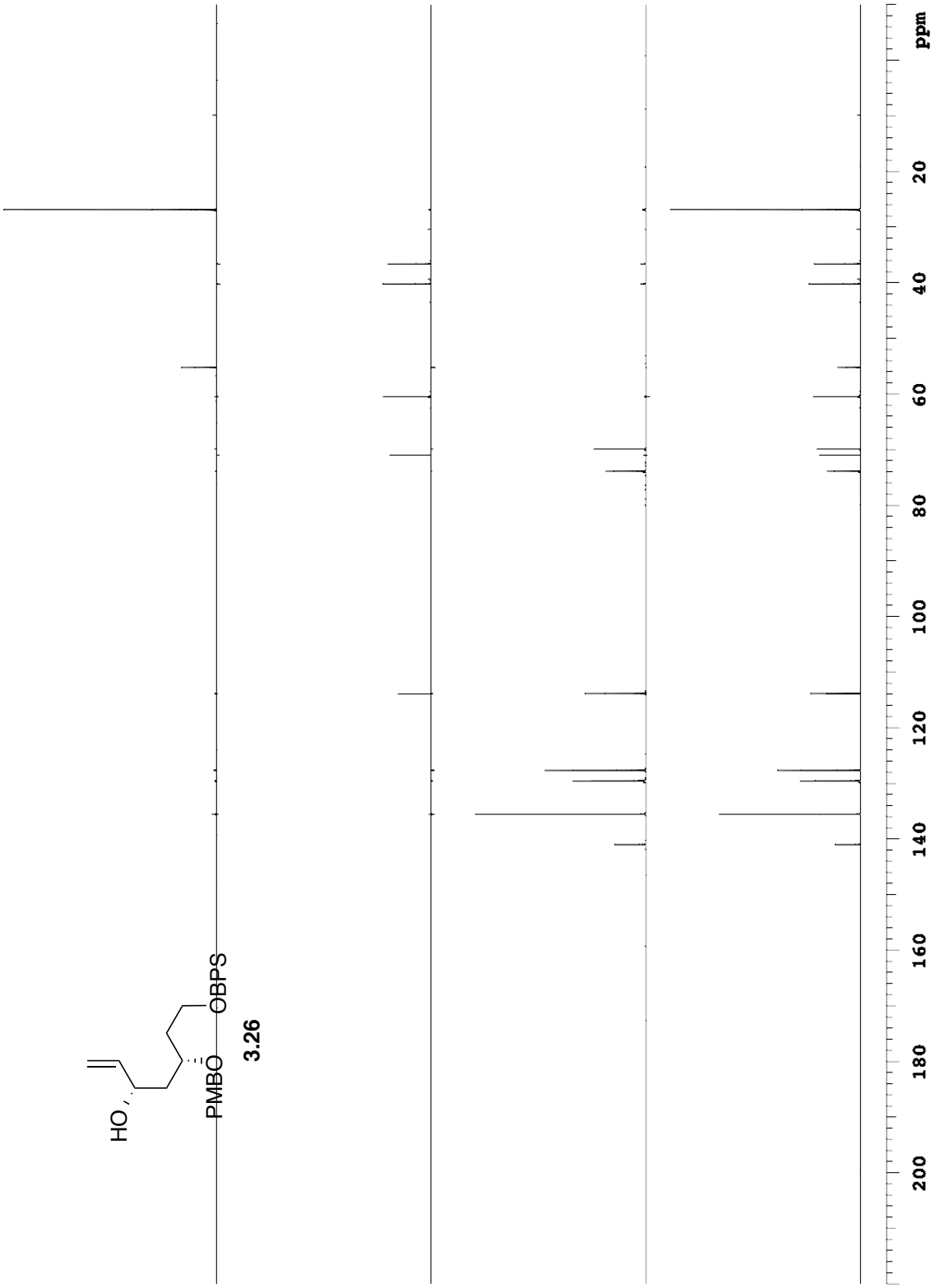

OMe
O
BPSO
Me
Me
MeO
O
TBSO
3.10
ppm
1
2
3
4
5
6
7
8
9

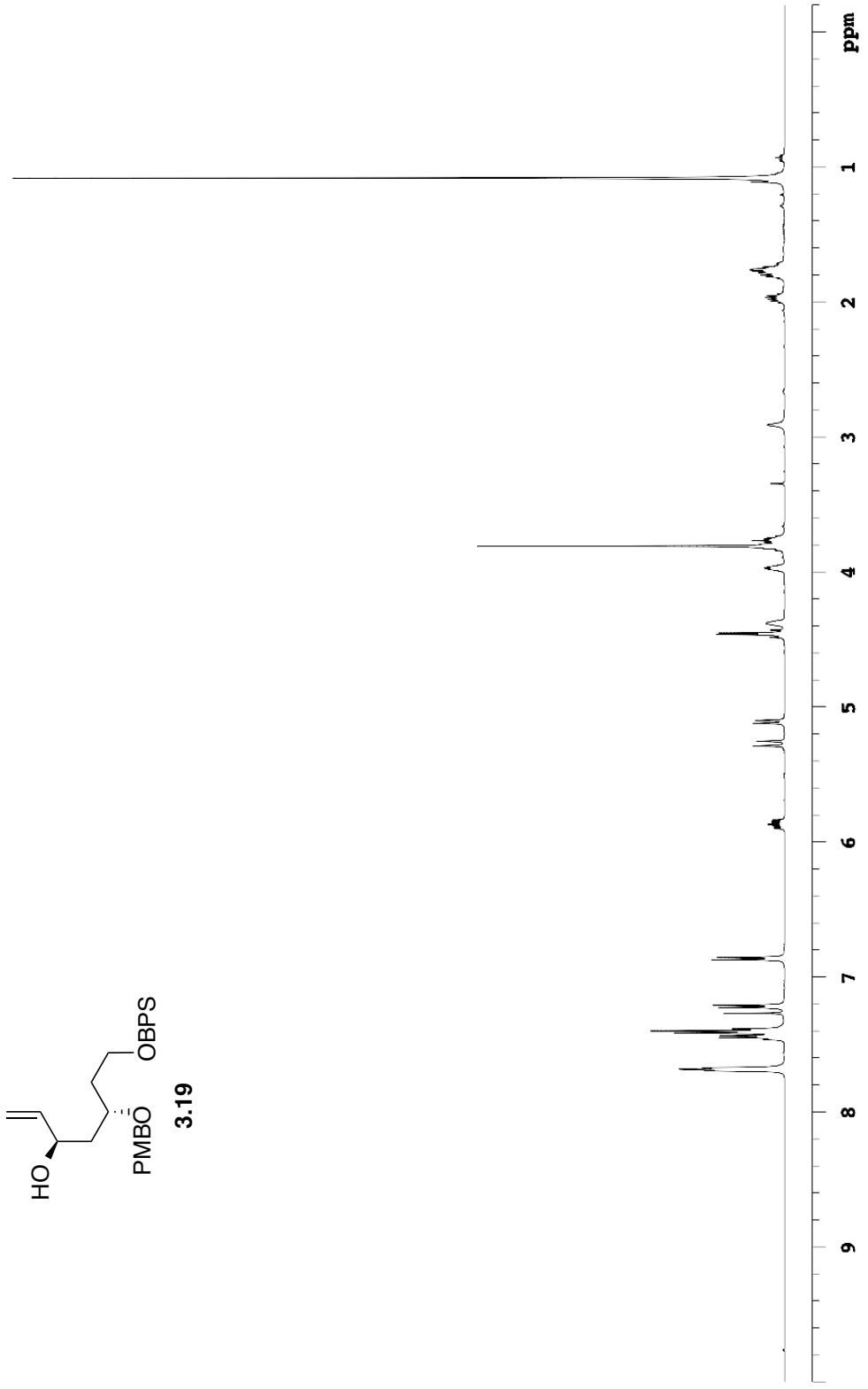

OMe
O
BPSO
Me
Me
MeO
O
TBSO
3.10
ppm
200
180
160
140
120
100
80
60
40
20

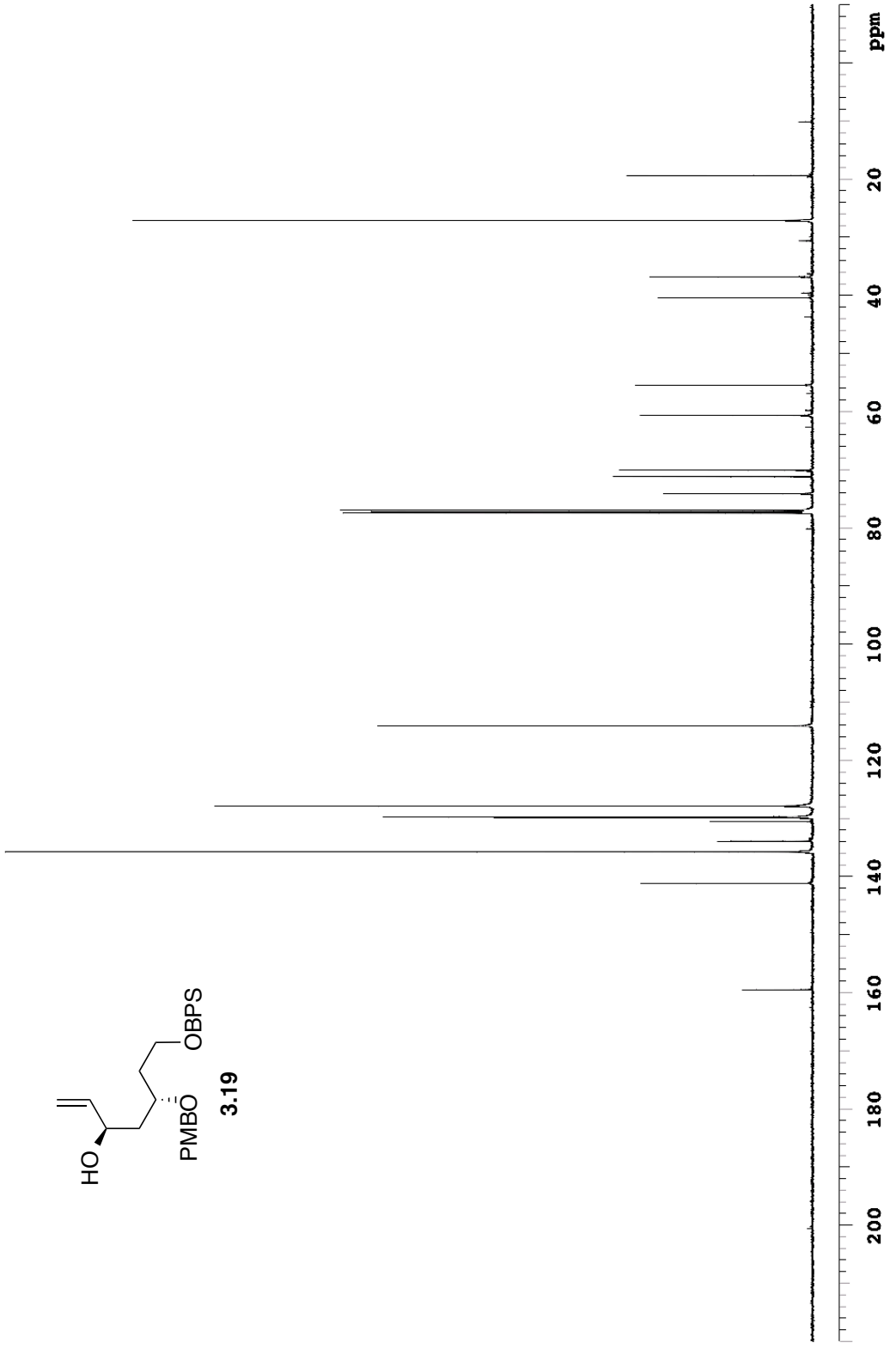

Me
Me
MeO
O
TBSO
OMe
O
BPSO
3.10
ppm
200
180
160
140
120
100
80
60
40
20

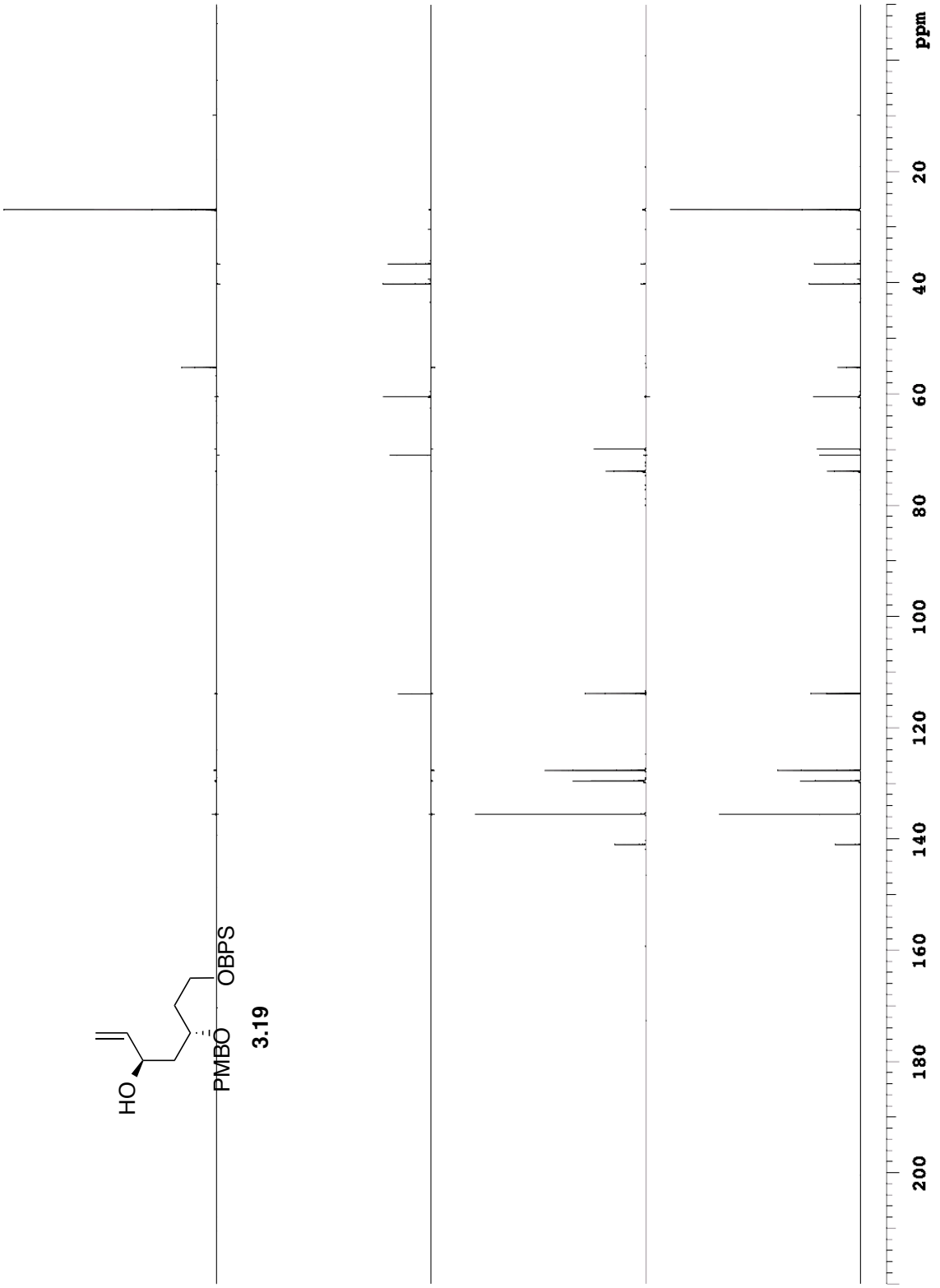

HO
PMBO
OBPS
3.26
ppm
1
2
3
4
5
6
7
8
9

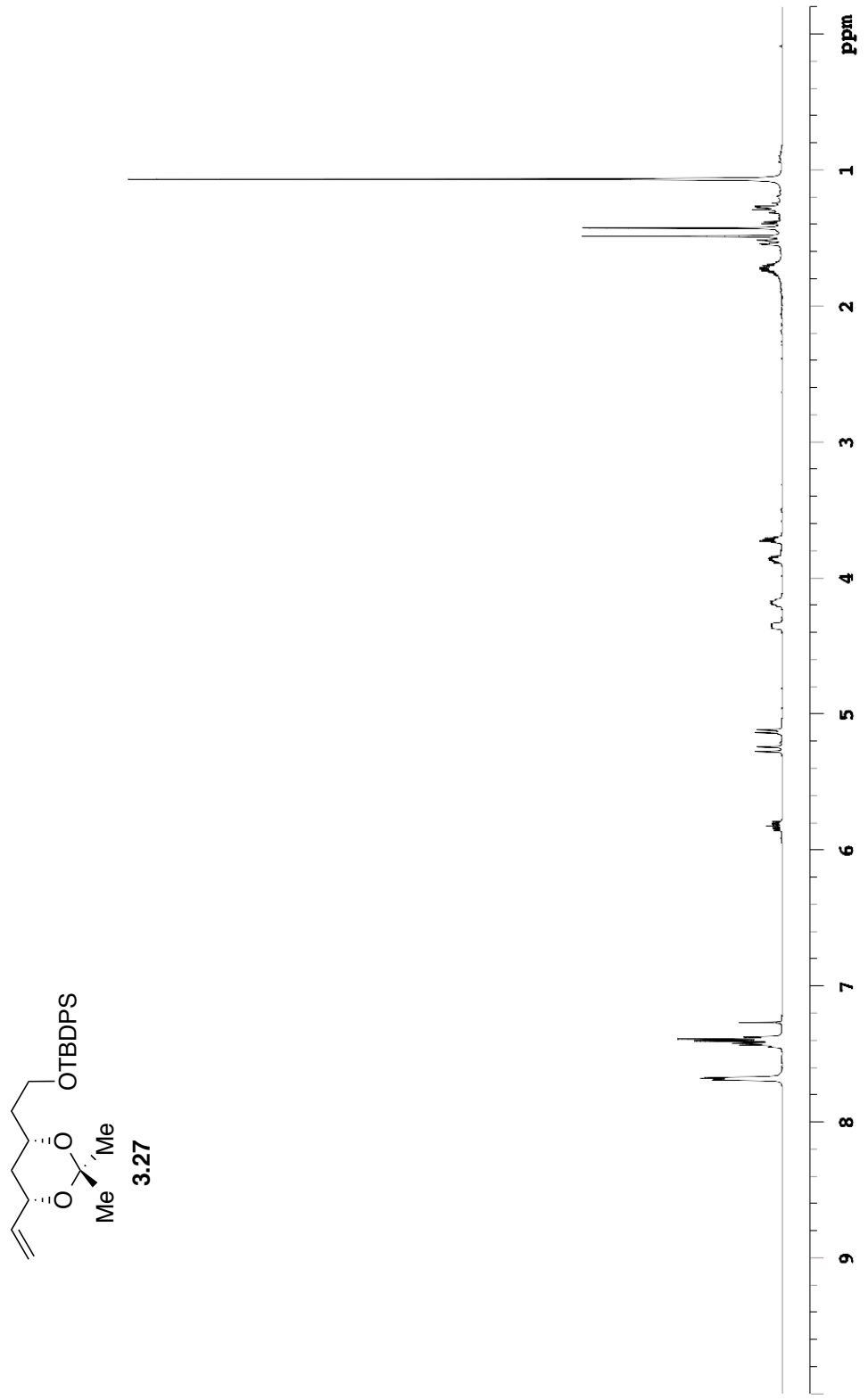

HO
PMBO
OBPS
3.26
ppm
200
180
160
140
120
100
80
60
40
20

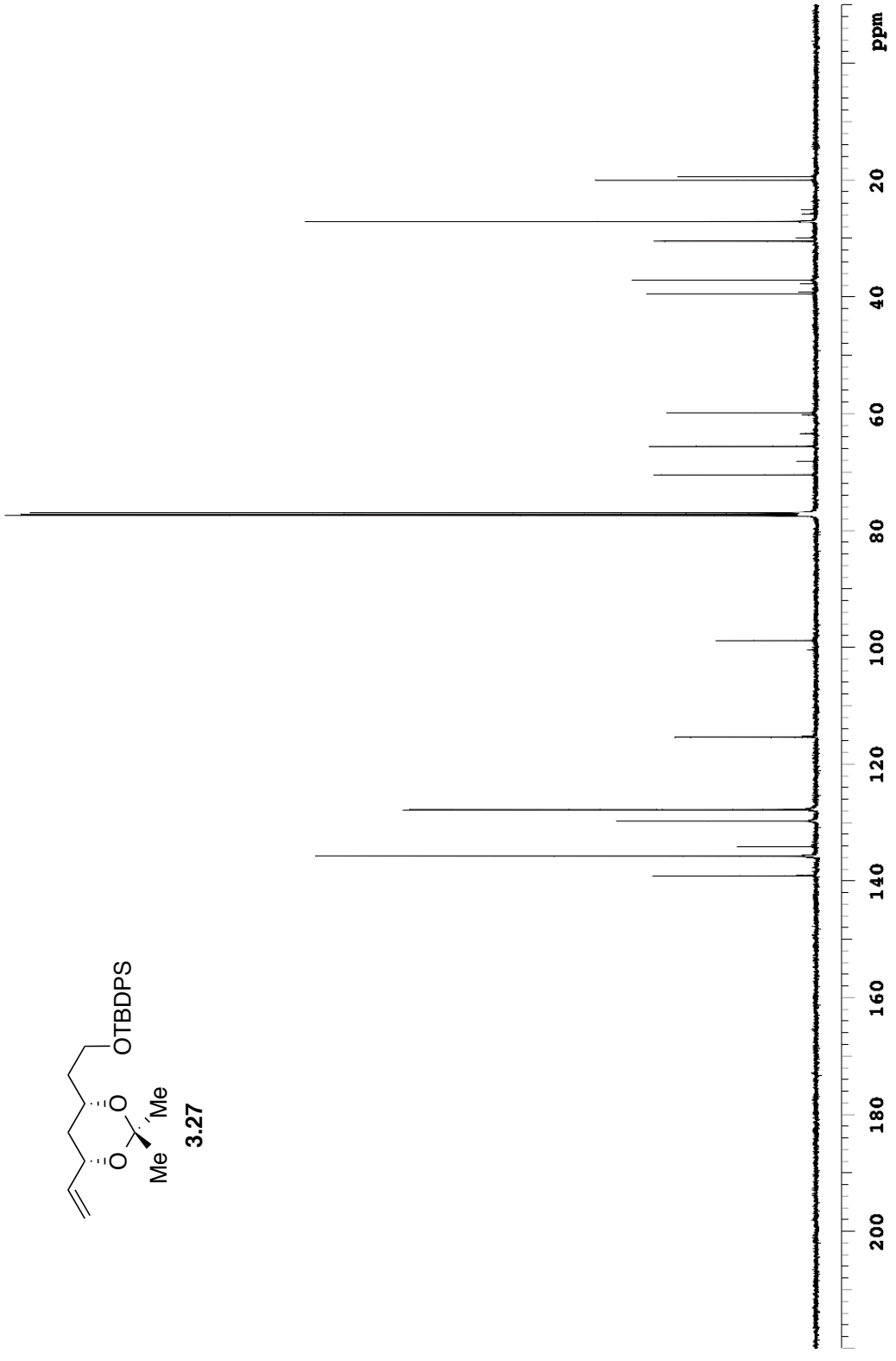

HO
PMBO
OBPS
3.26
ppm
200
180
160
140
120
100
80
60
40
20

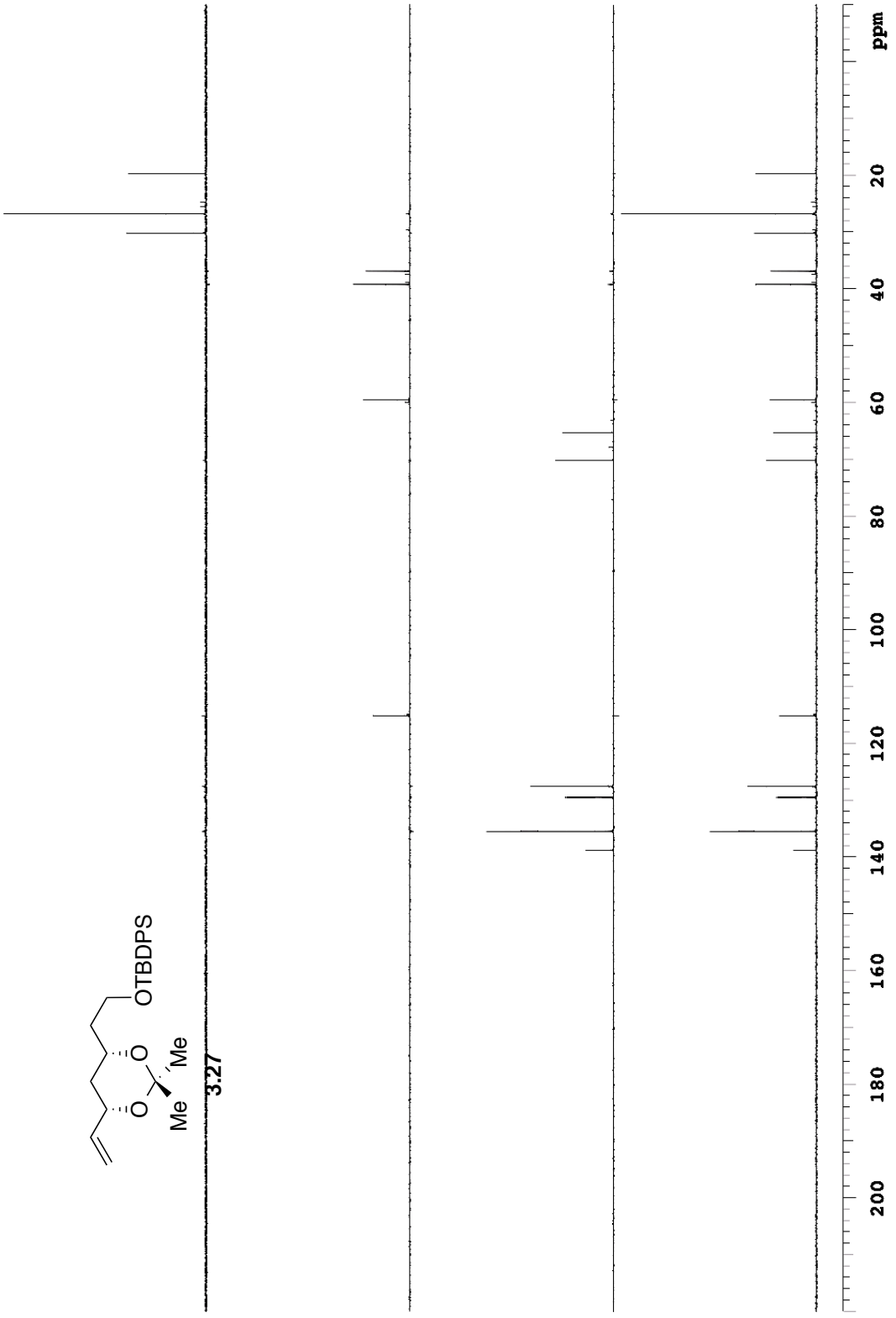

HO
PMBO
OBPS
3.19
ppm
1
2
3
4
5
6
7
8
9

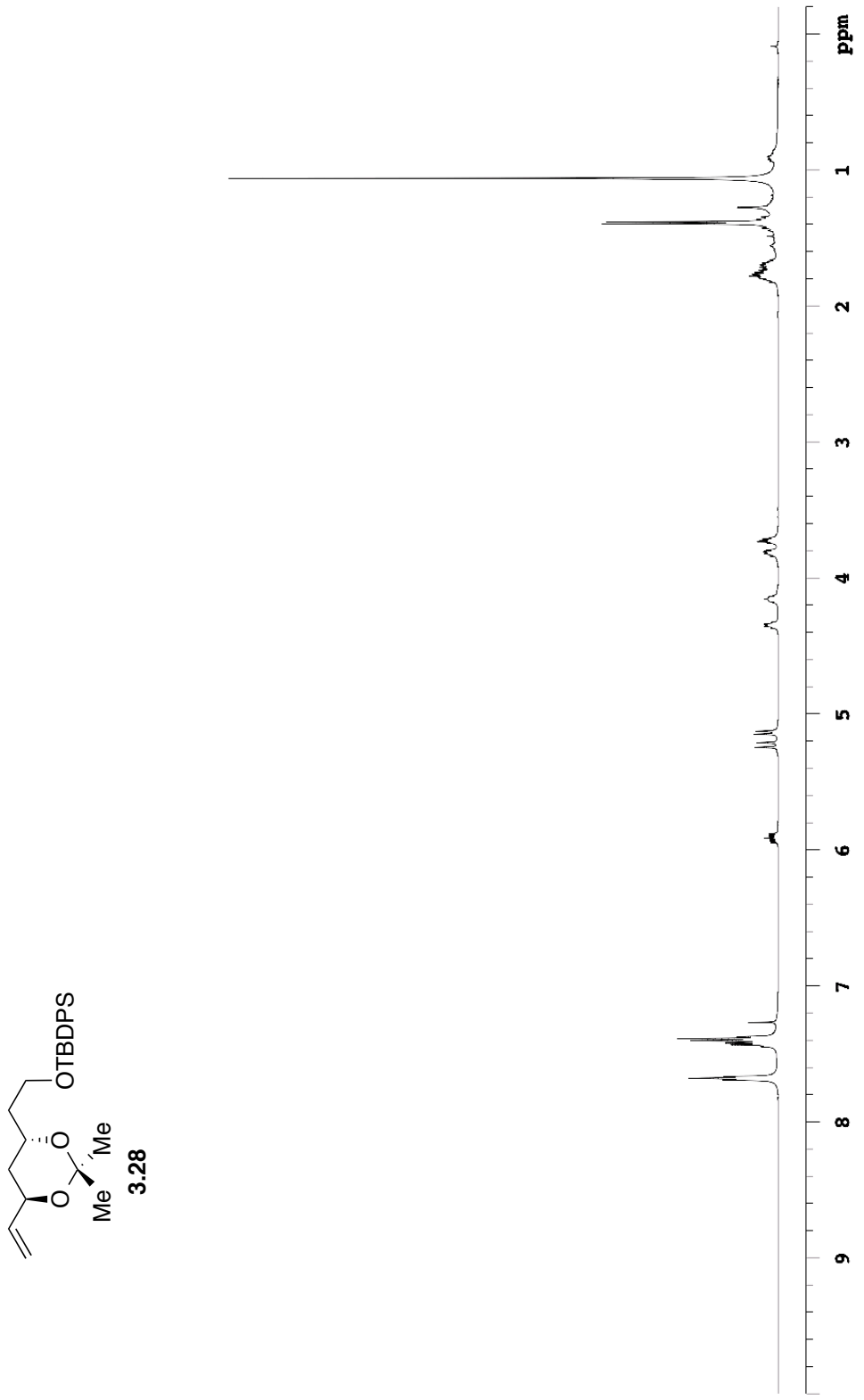

HO
PMBO
OBPS
3.19
ppm
20
40
60
80
100
120
140
160
180
200

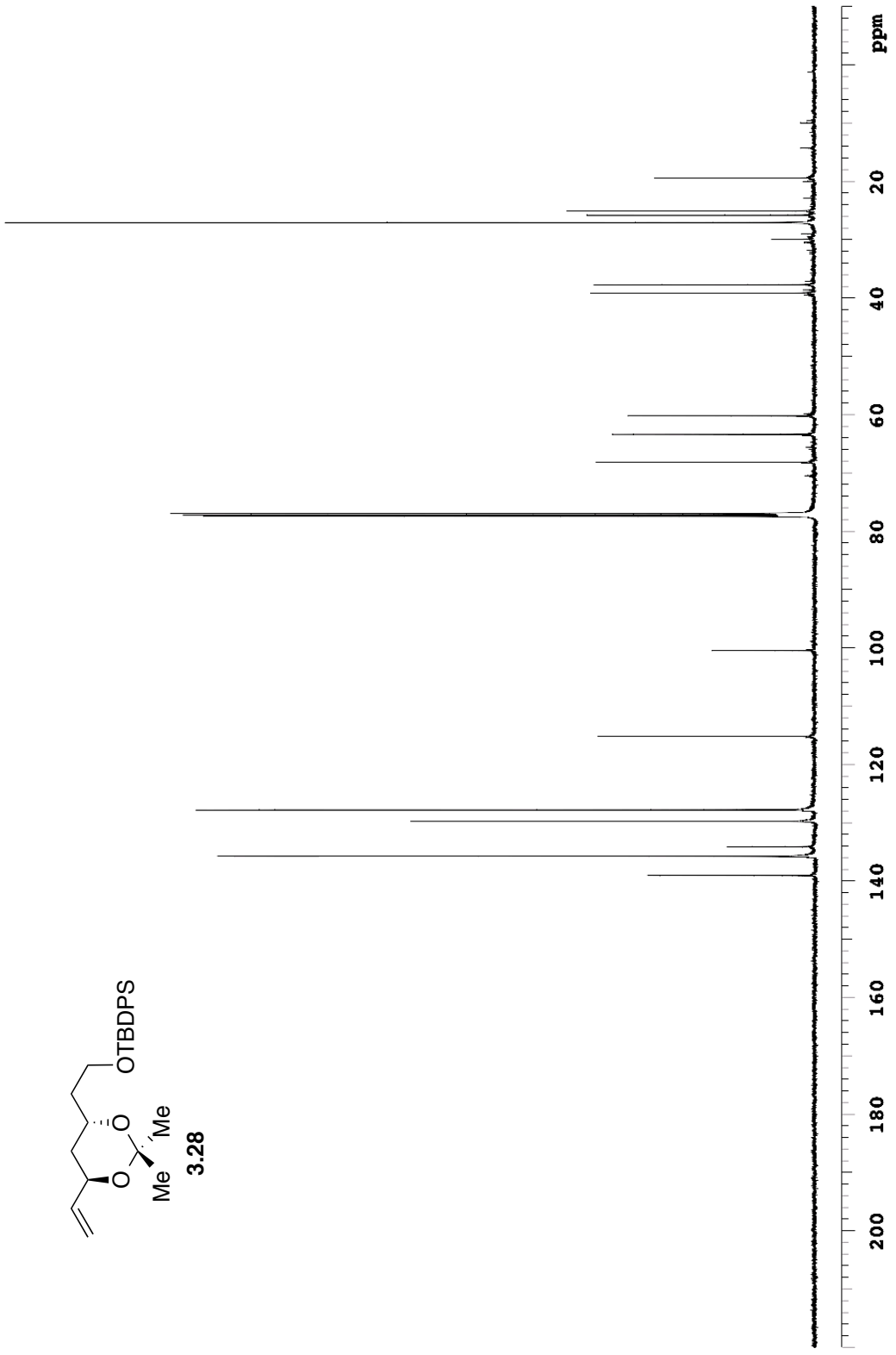

HO
PMBO
OBPS
3.19
ppm
200
180
160
140
120
100
80
60
40
20

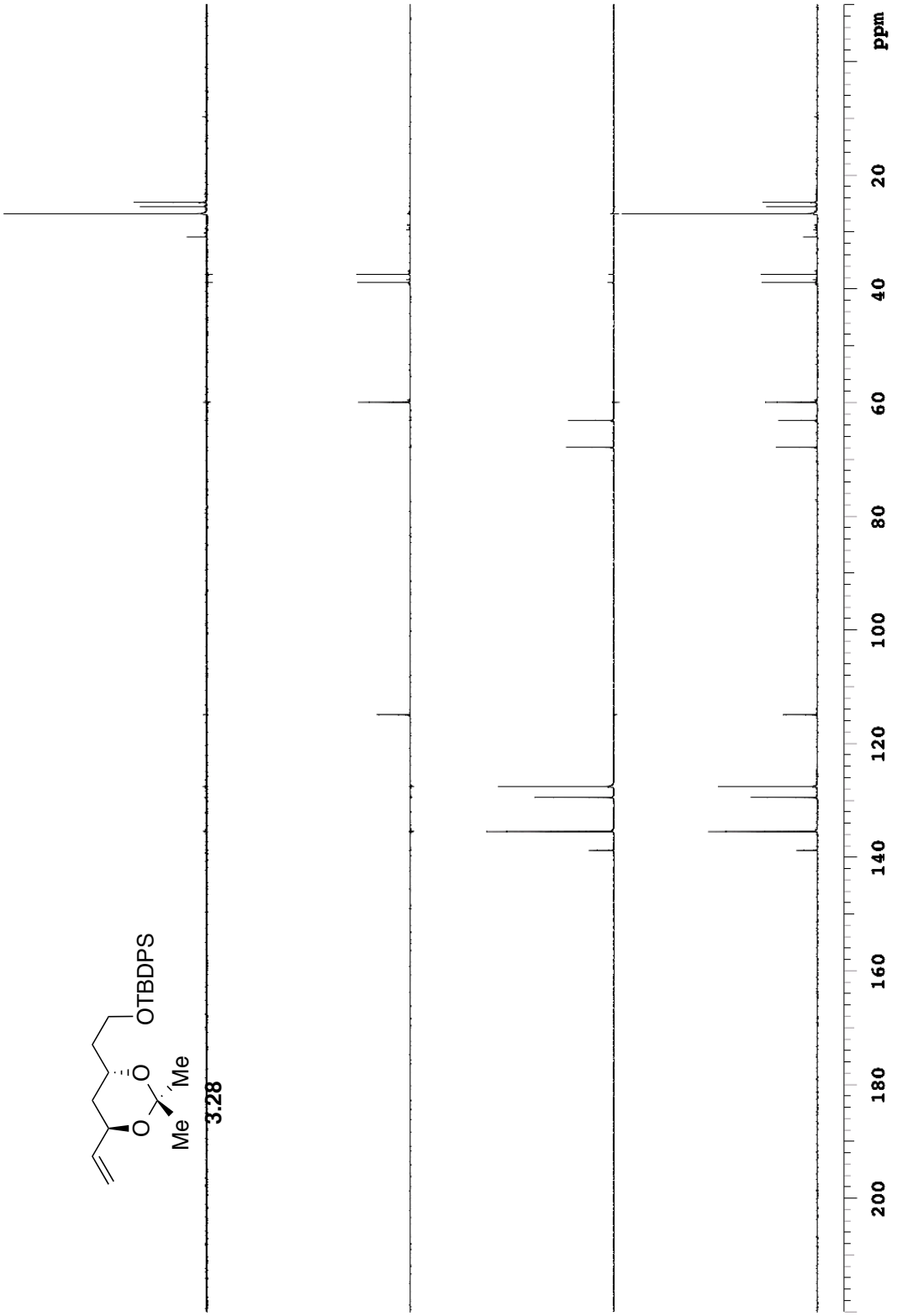

OTBDPS
O
O
Me
Me
3.27
ppm
1
2
3
4
5
6
7
8
9

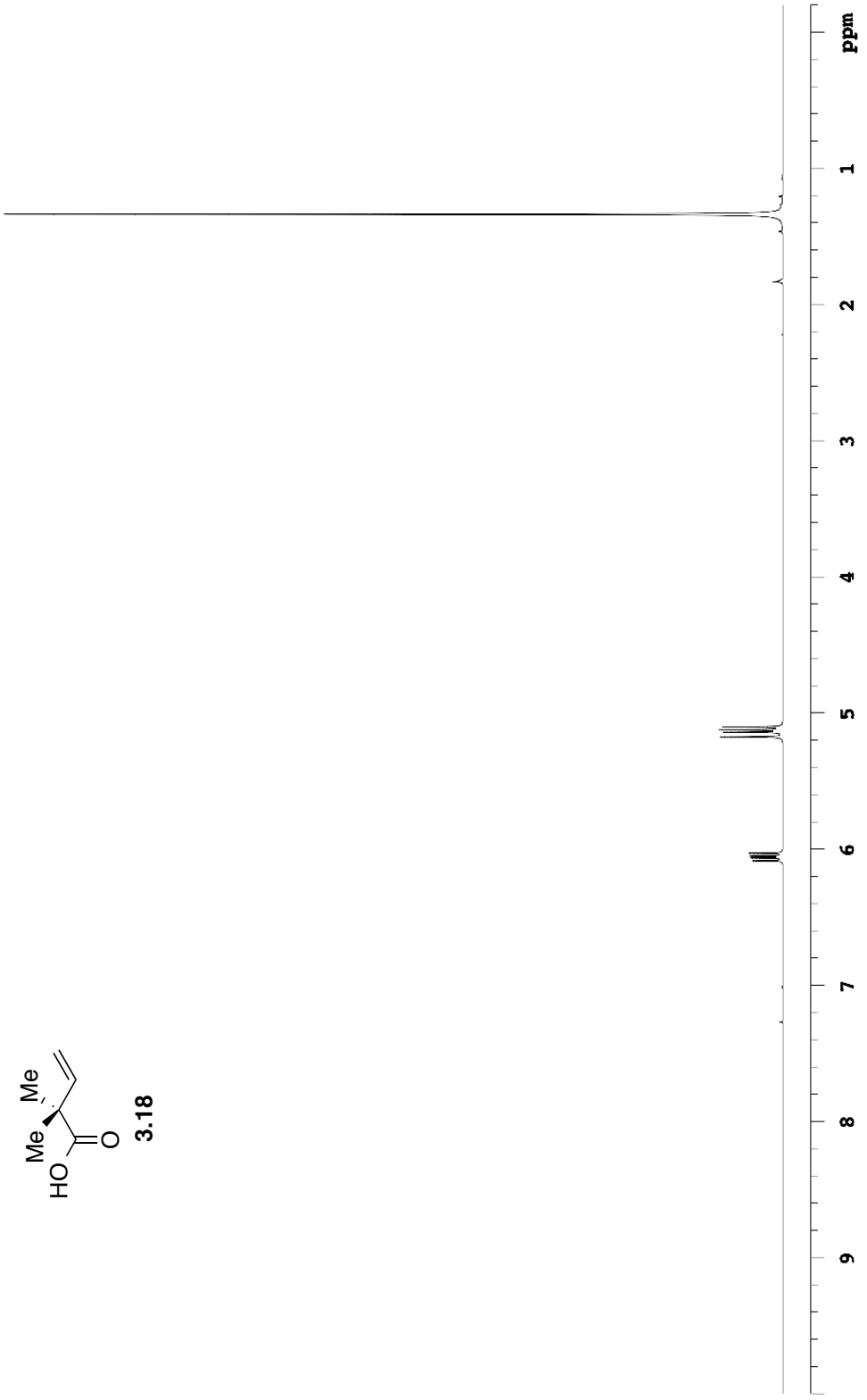

OTBDPS
O
O
Me
Me
3.27
ppm
200
180
160
140
120
100
80
60
40
20

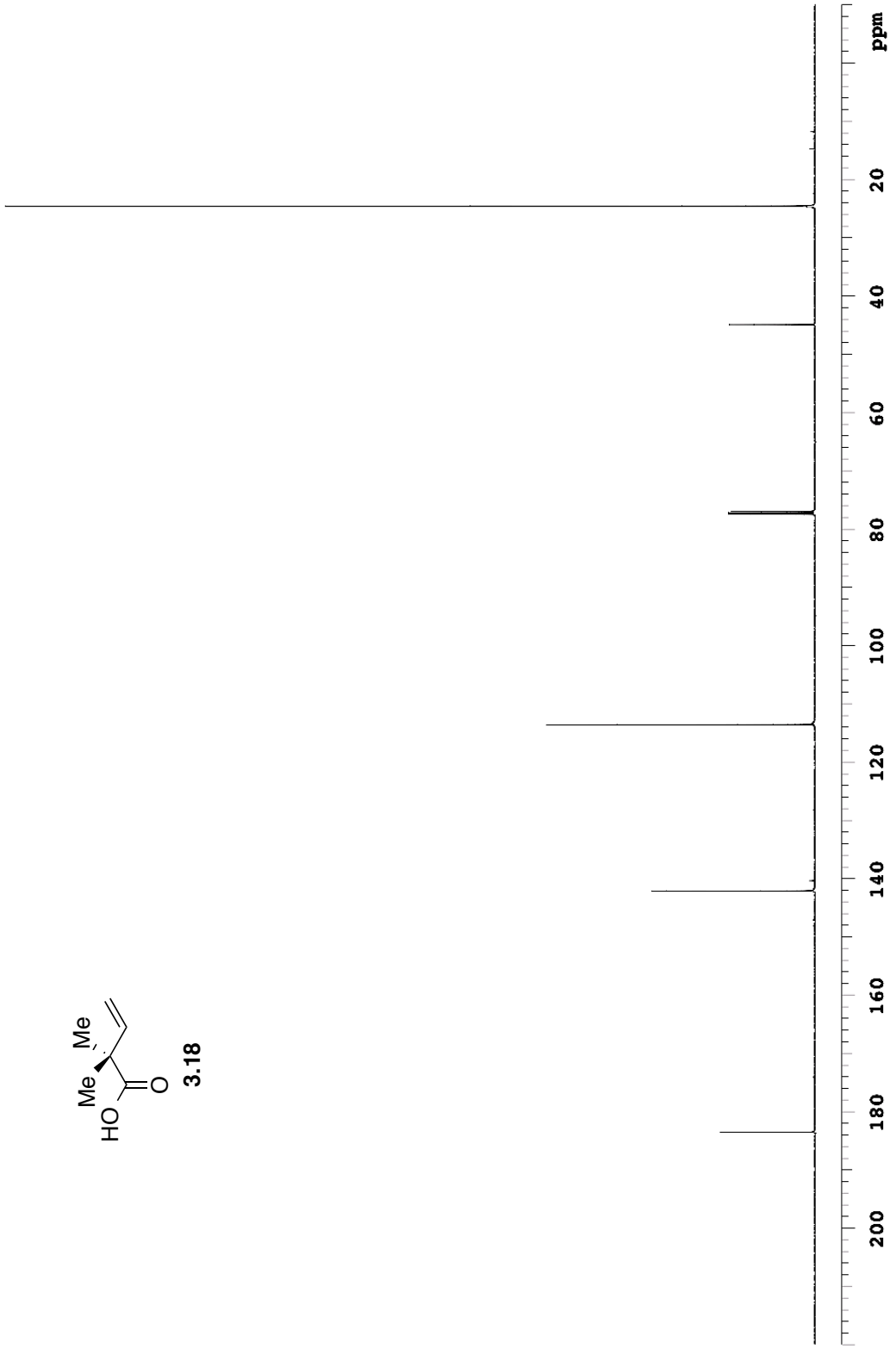

OTBDPS
O
O
Me
Me
3.27
200
180
160
140
120
100
80
60
40
20
ppm

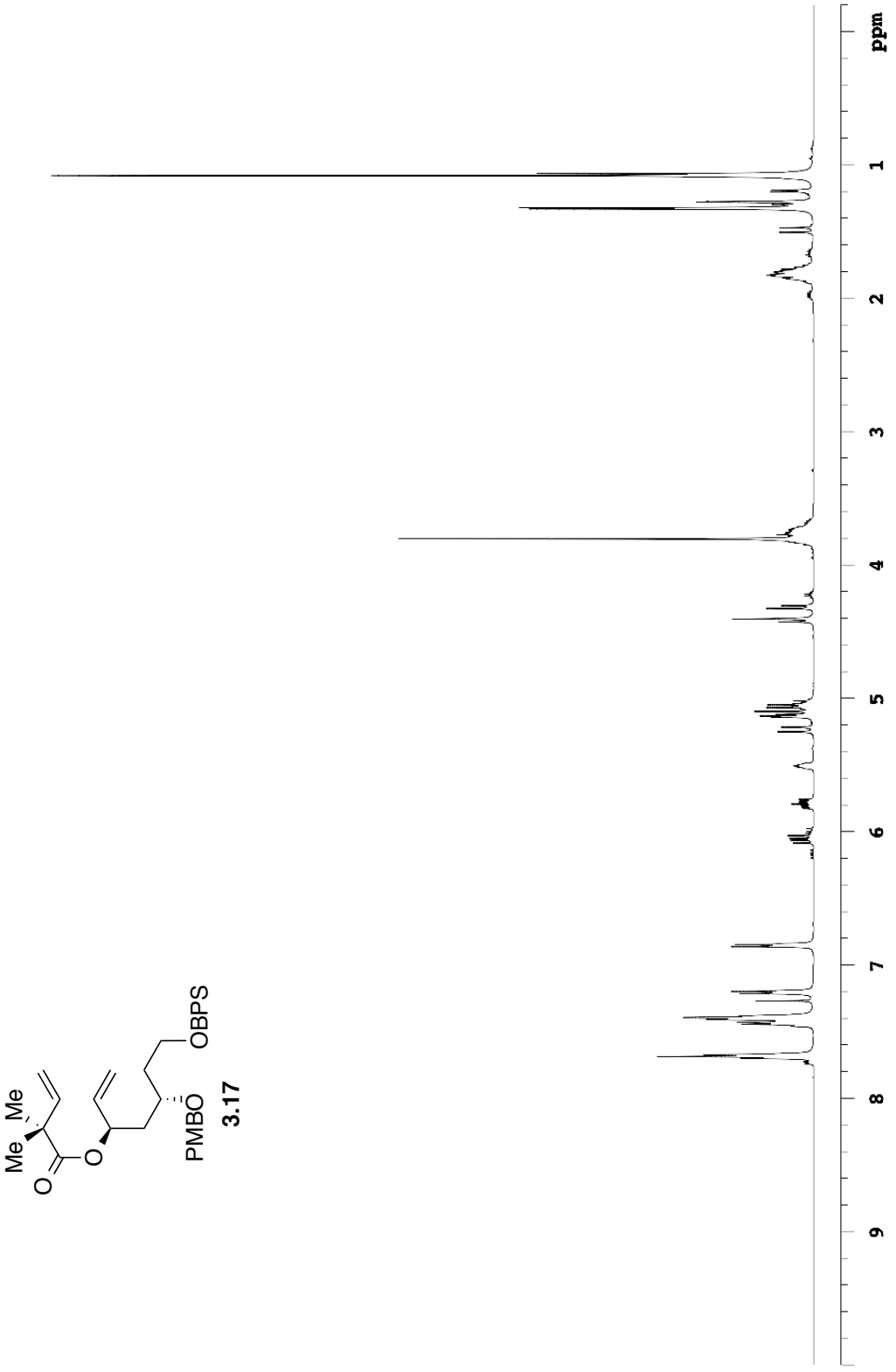

OTBDPS
O
O
O
Me
Me
3.28
ppm
1
2
3
4
5
6
7
8
9

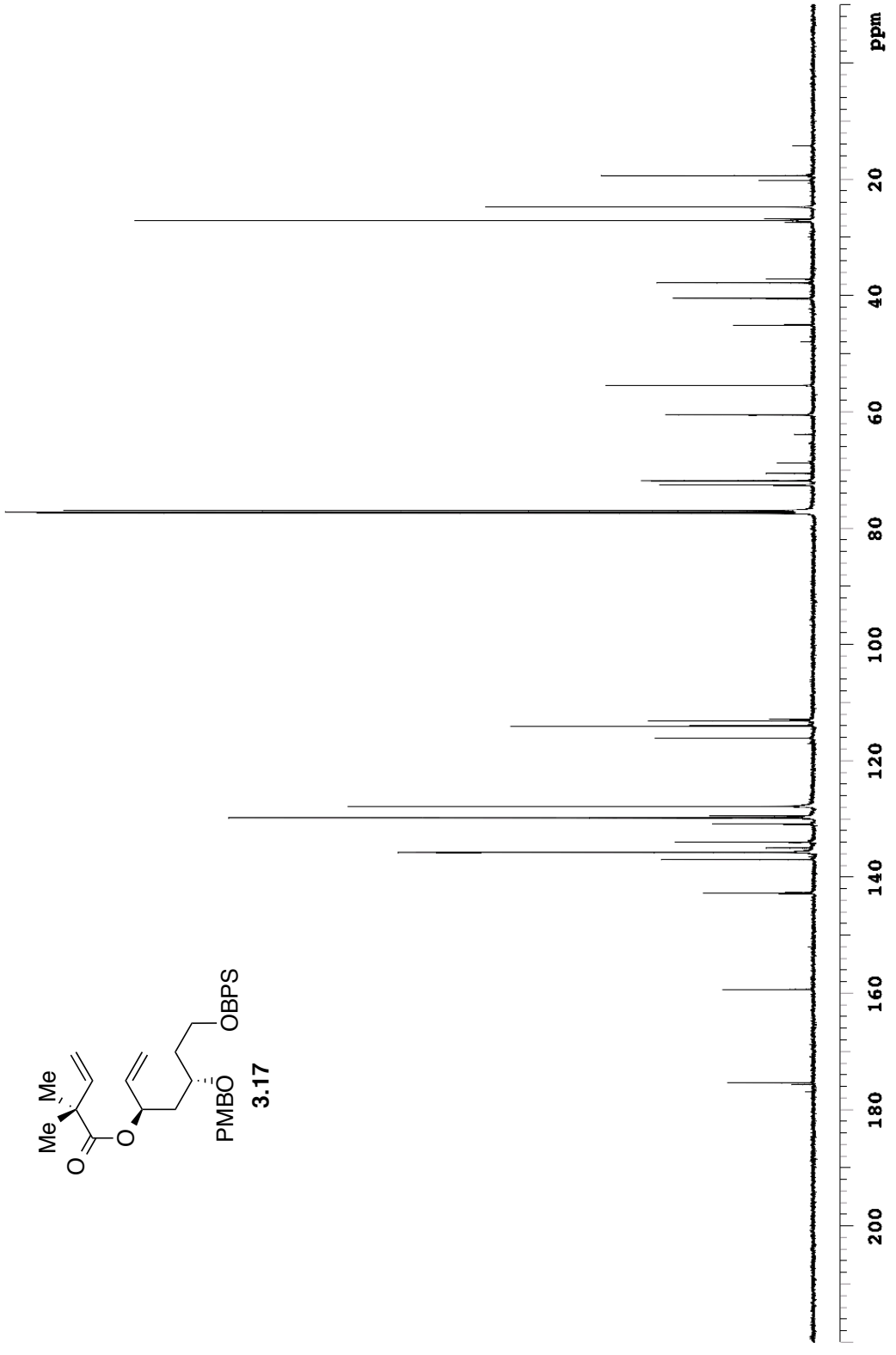

OTBDPS
O
O
Me
Me
3.28
ppm
200
180
160
140
120
100
80
60
40
20

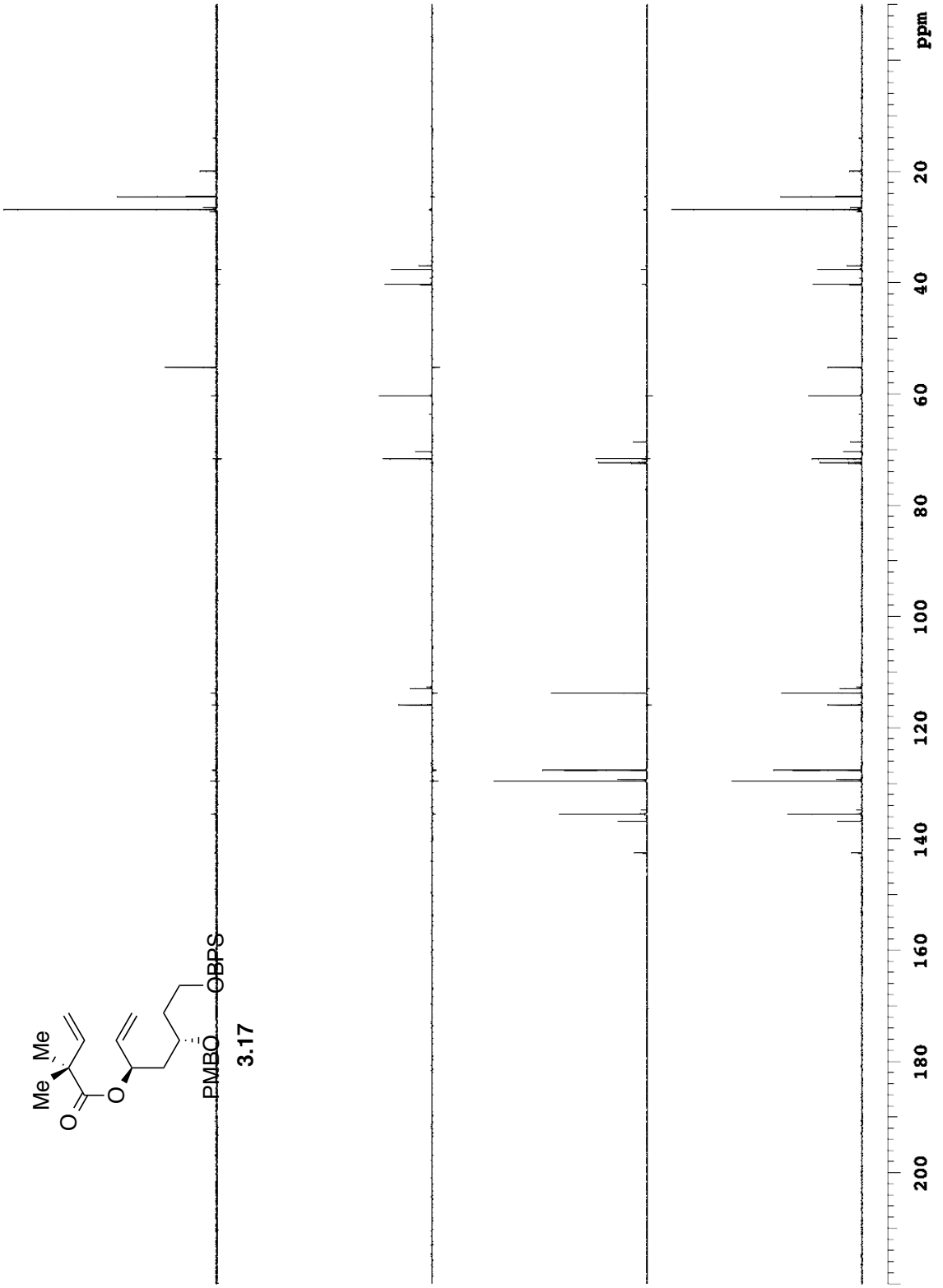

OTBDPS
O
O
Me
Me
3.28
ppm
200
180
160
140
120
100
80
60
40
20

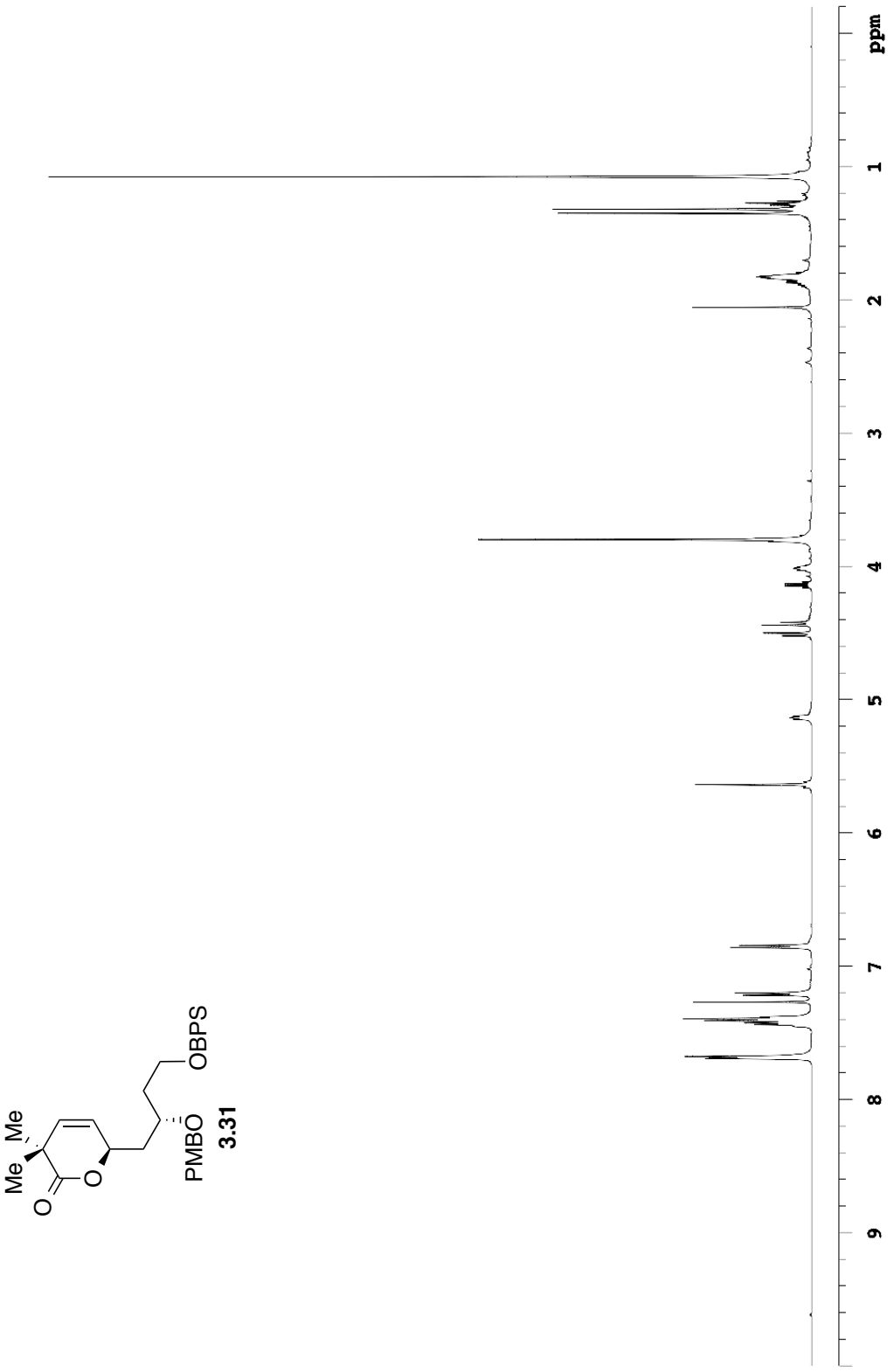

Me
Me
HO
O
3.18
ppm
1
2
3
4
5
6
7
8
9

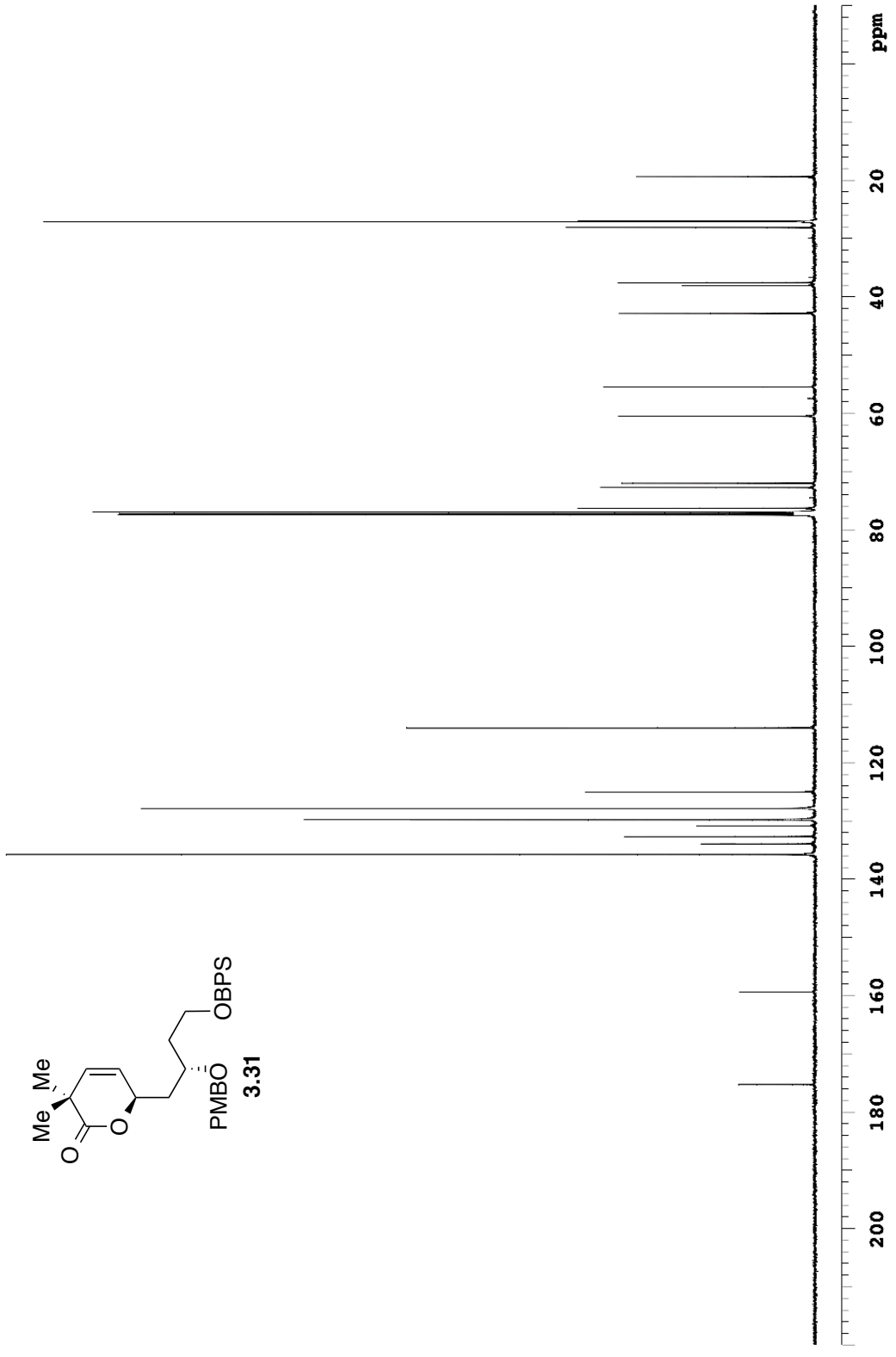

Me
Me
HO
O
3.18
200
180
160
140
120
100
80
60
40
20
ppm

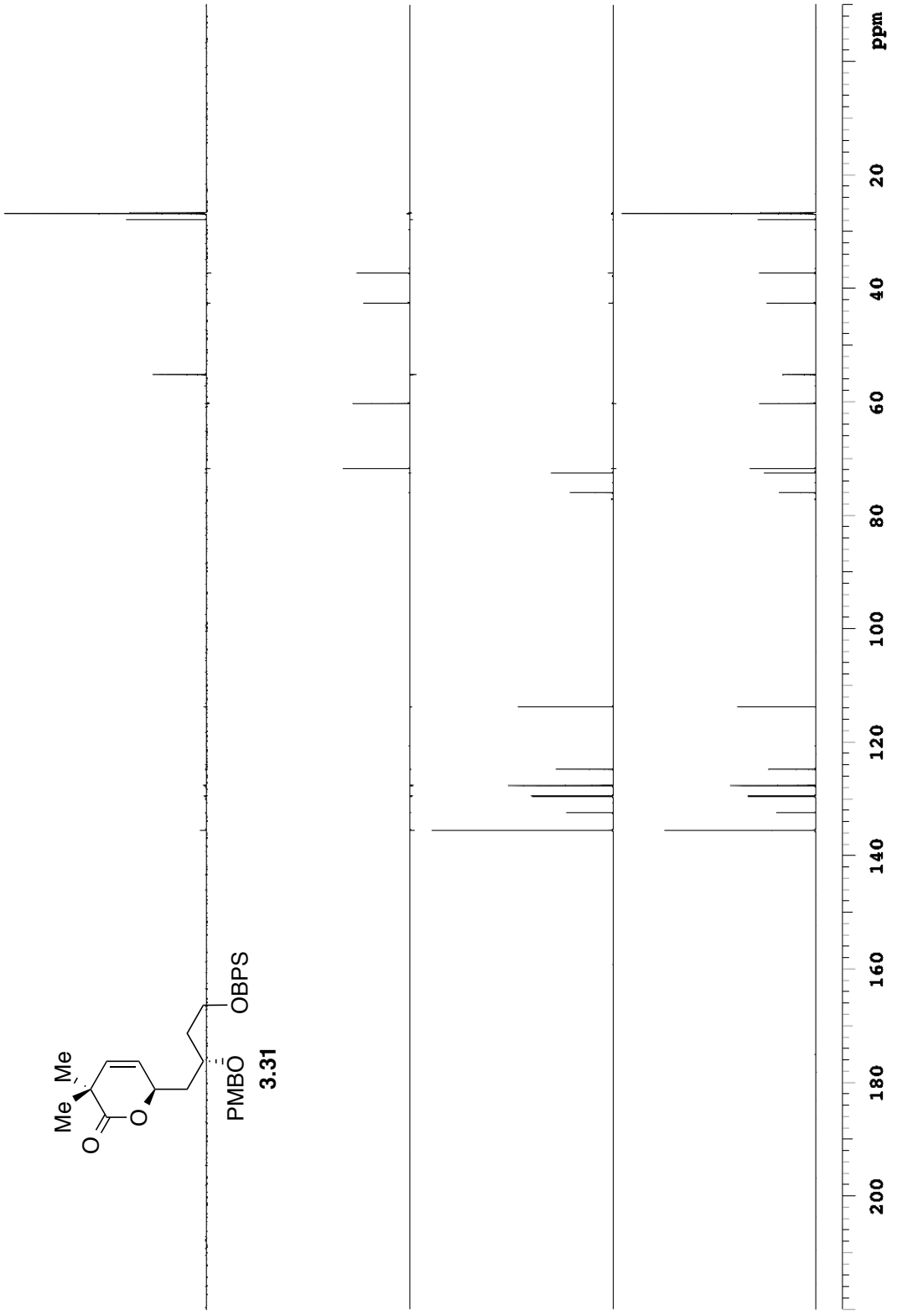

Me
Me
O
O
PMBO
OBPS
3.17
ppm
1
2
3
4
5
6
7
8
9

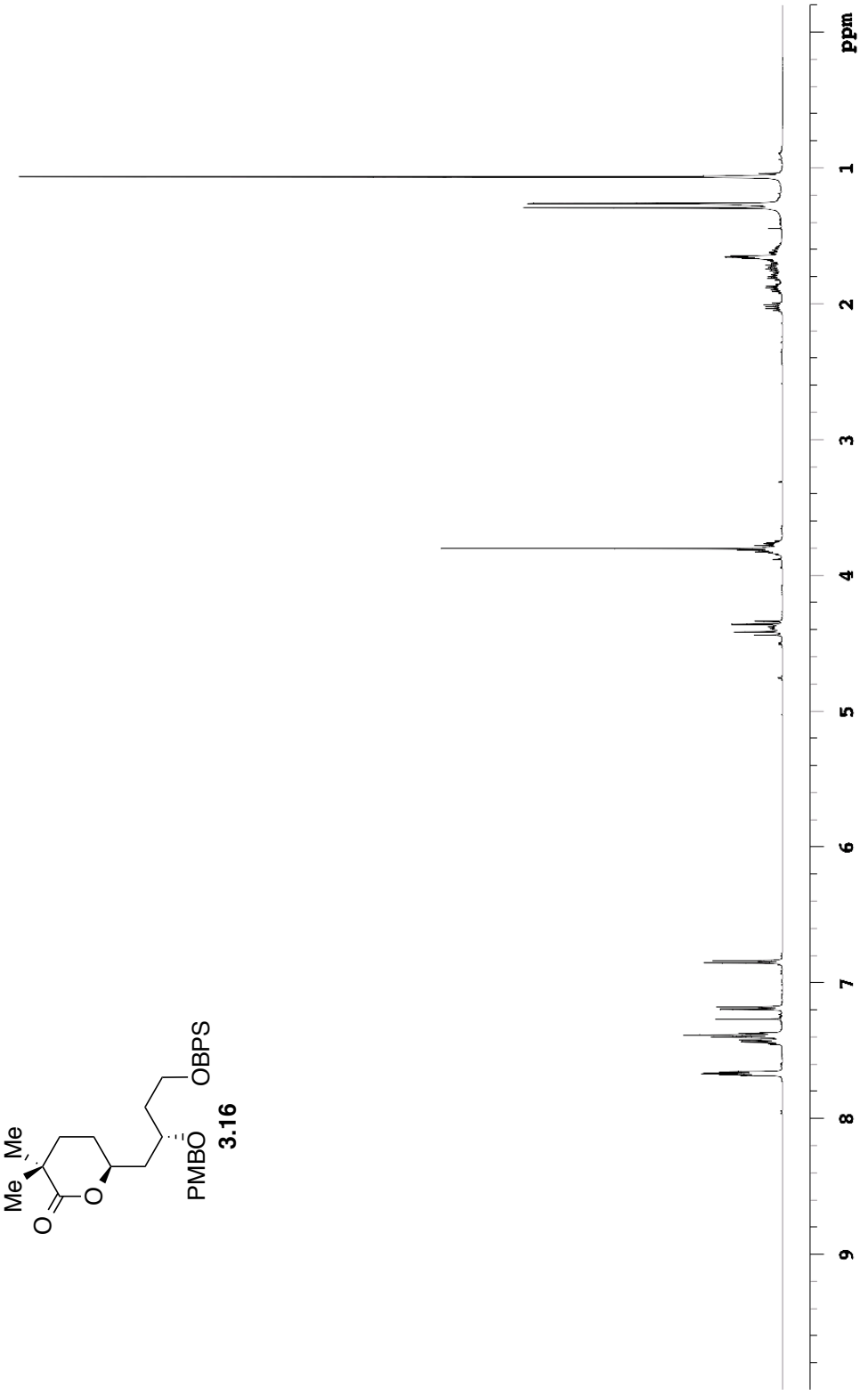

Me
Me
O
O
PMBO
OBPS
3.17
ppm
20
40
60
80
100
120
140
160
180
200

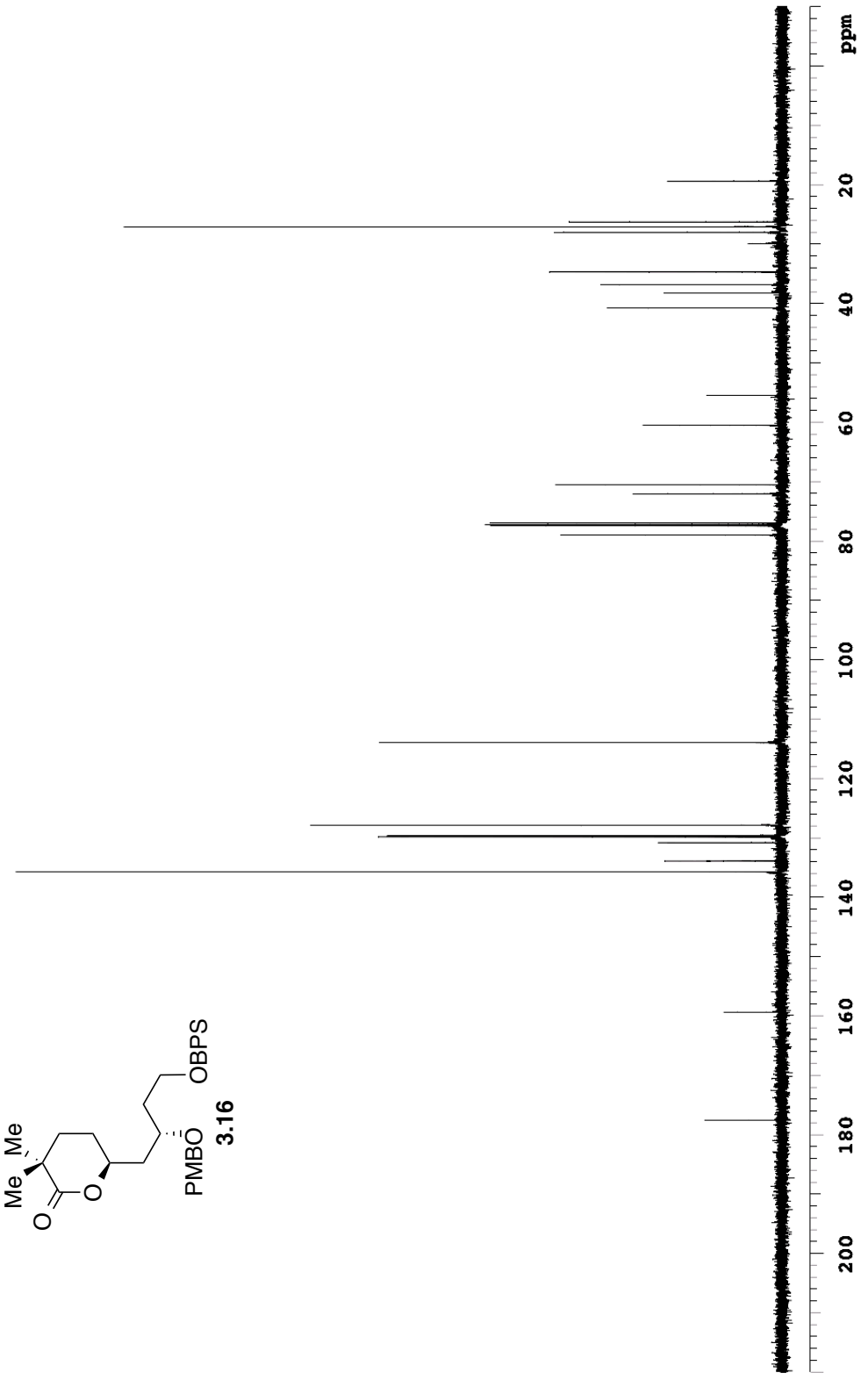

Me
Me
O
O
PMBO
OBPS
3.17
ppm
200
180
160
140
120
100
80
60
40
20

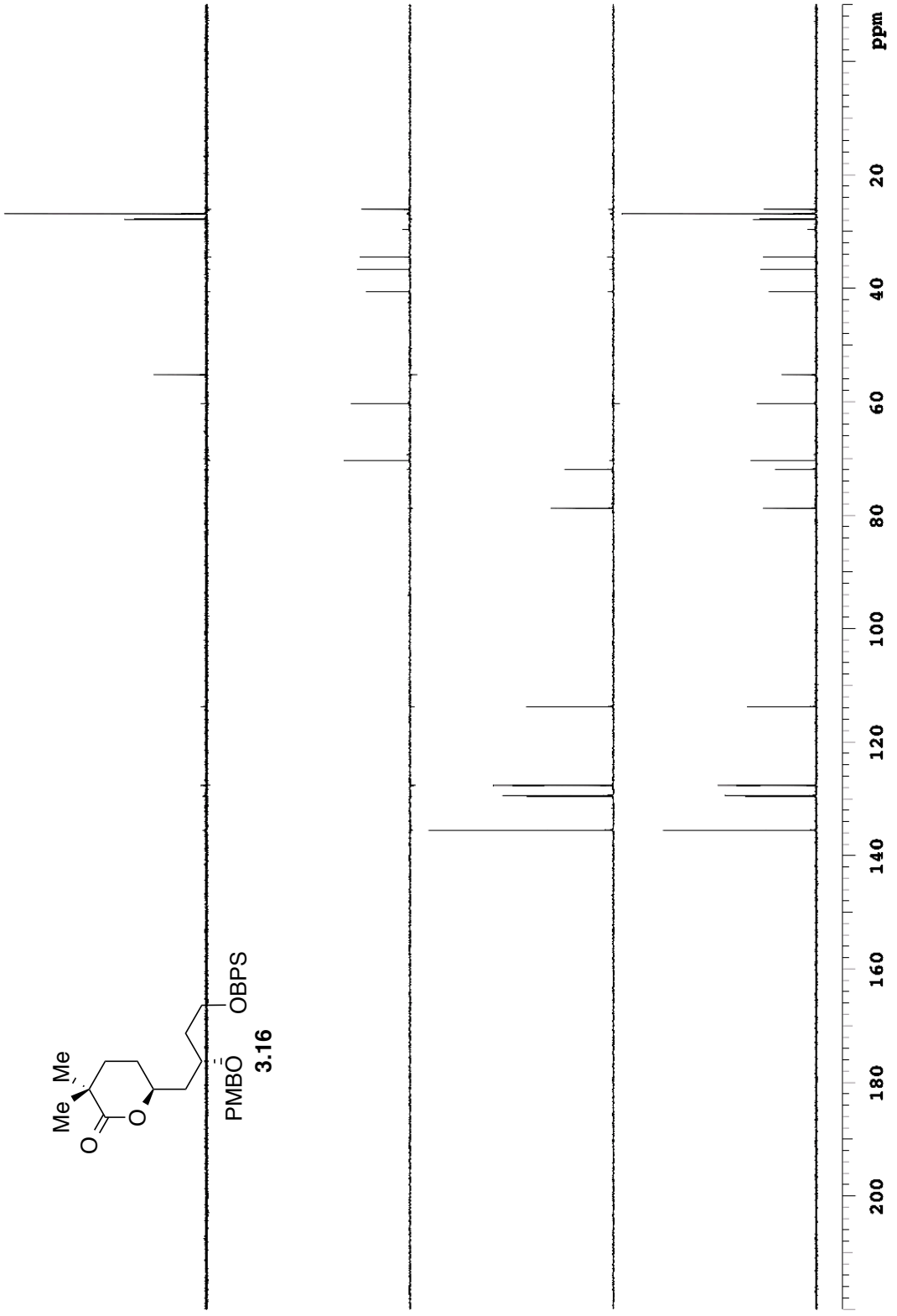

Me
Me
O
O
PMBO
OBPS
3.31
ppm
1
2
3
4
5
6
7
8
9

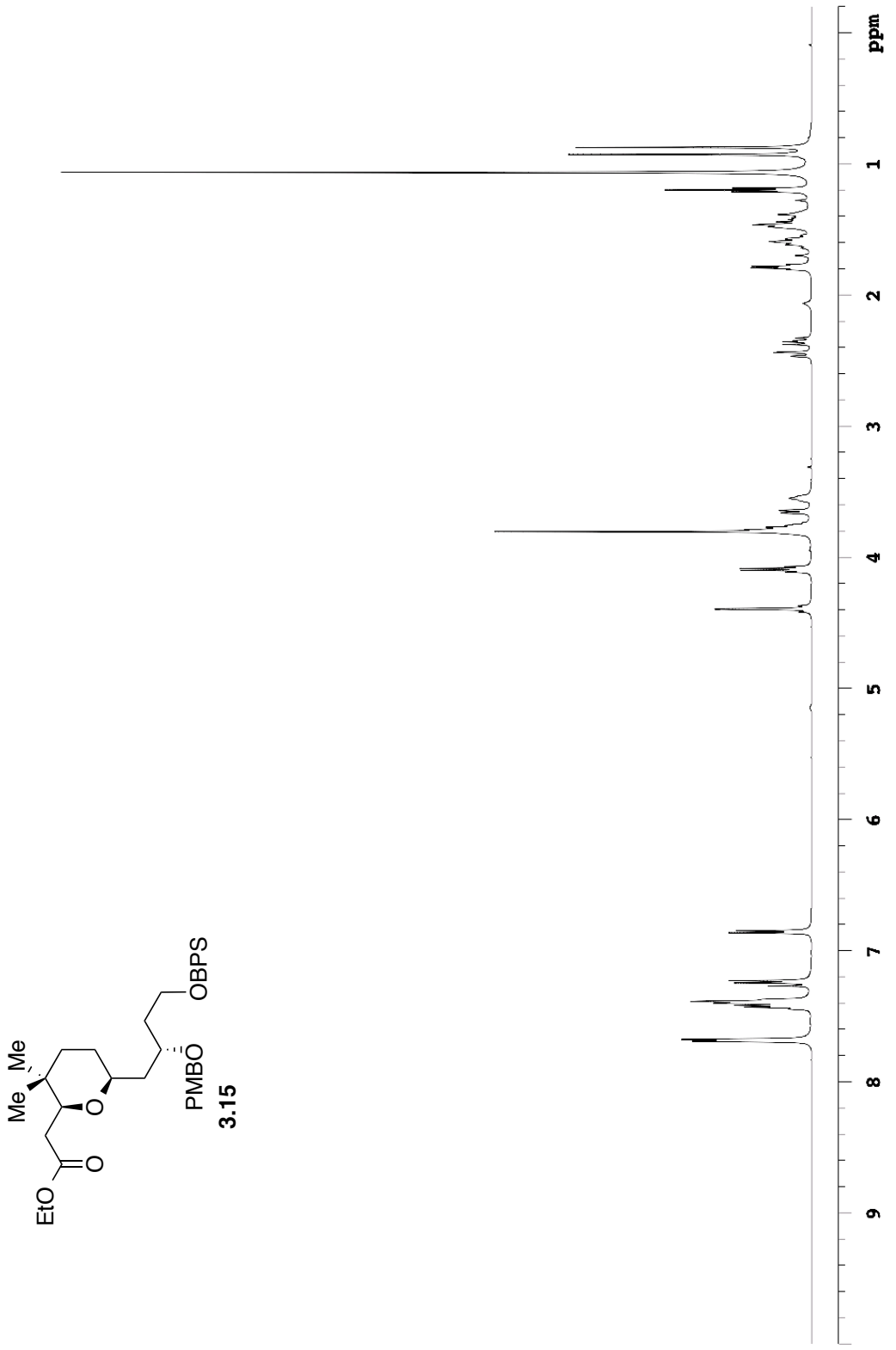

Me
Me
O
O
PMBO
OBPS
3.31
ppm
20
40
60
80
100
120
140
160
180
200

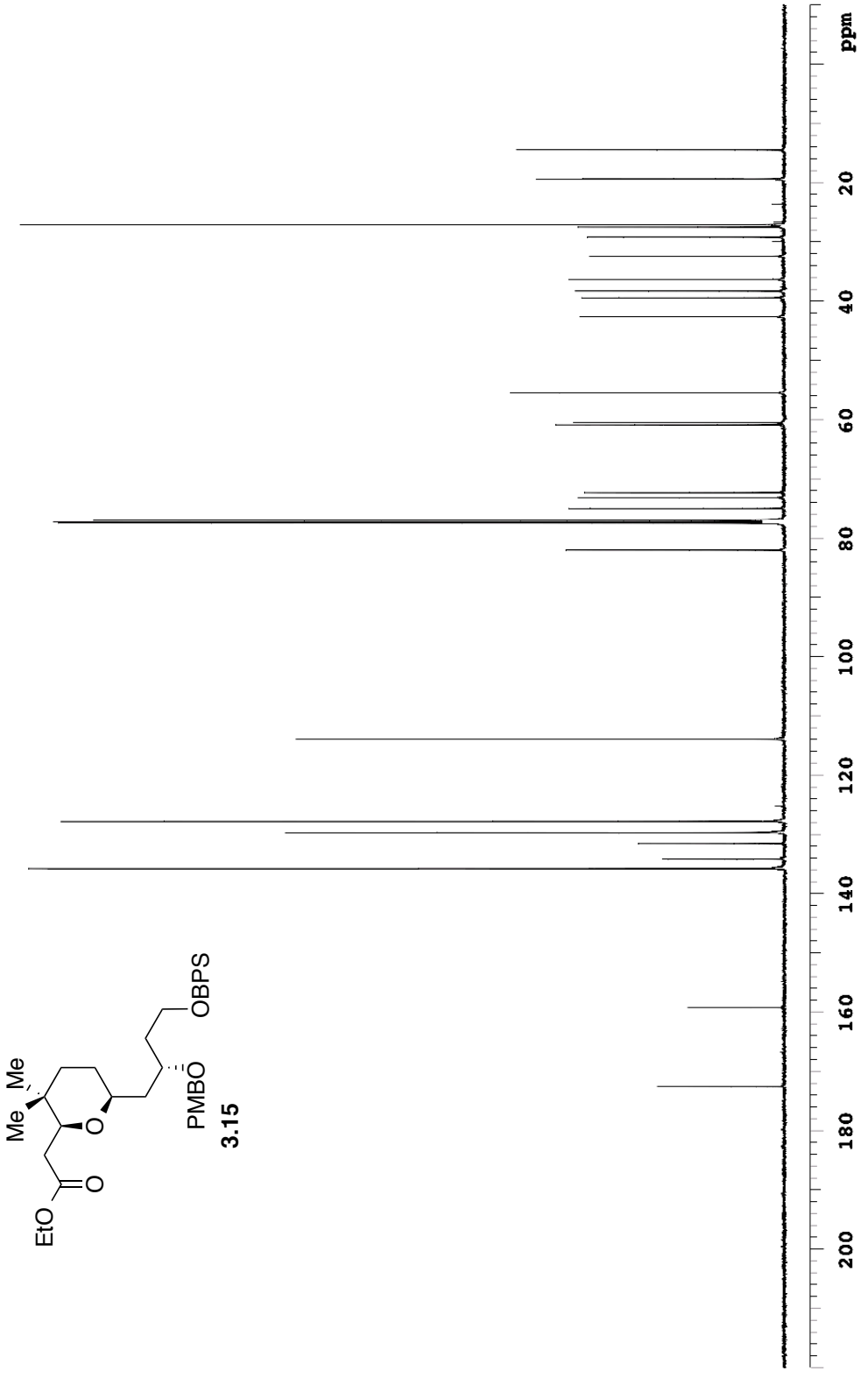

Me
Me
O
O
PMBO
OBPS
3.31
ppm
200
180
160
140
120
100
80
60
40
20

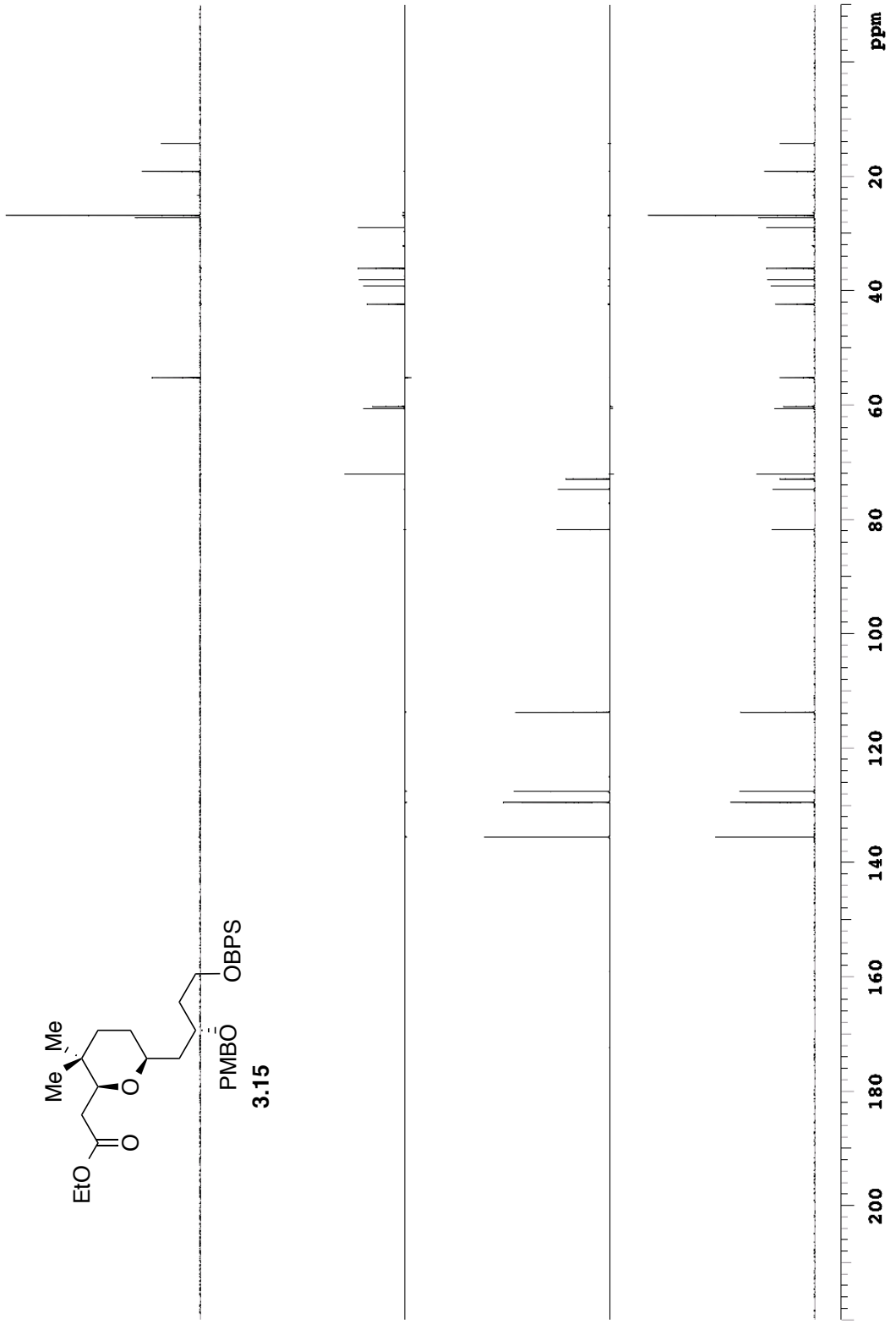

Me
Me
O
O
PMBO
OBPS
3.16
ppm
1
2
3
4
5
6
7
8
9

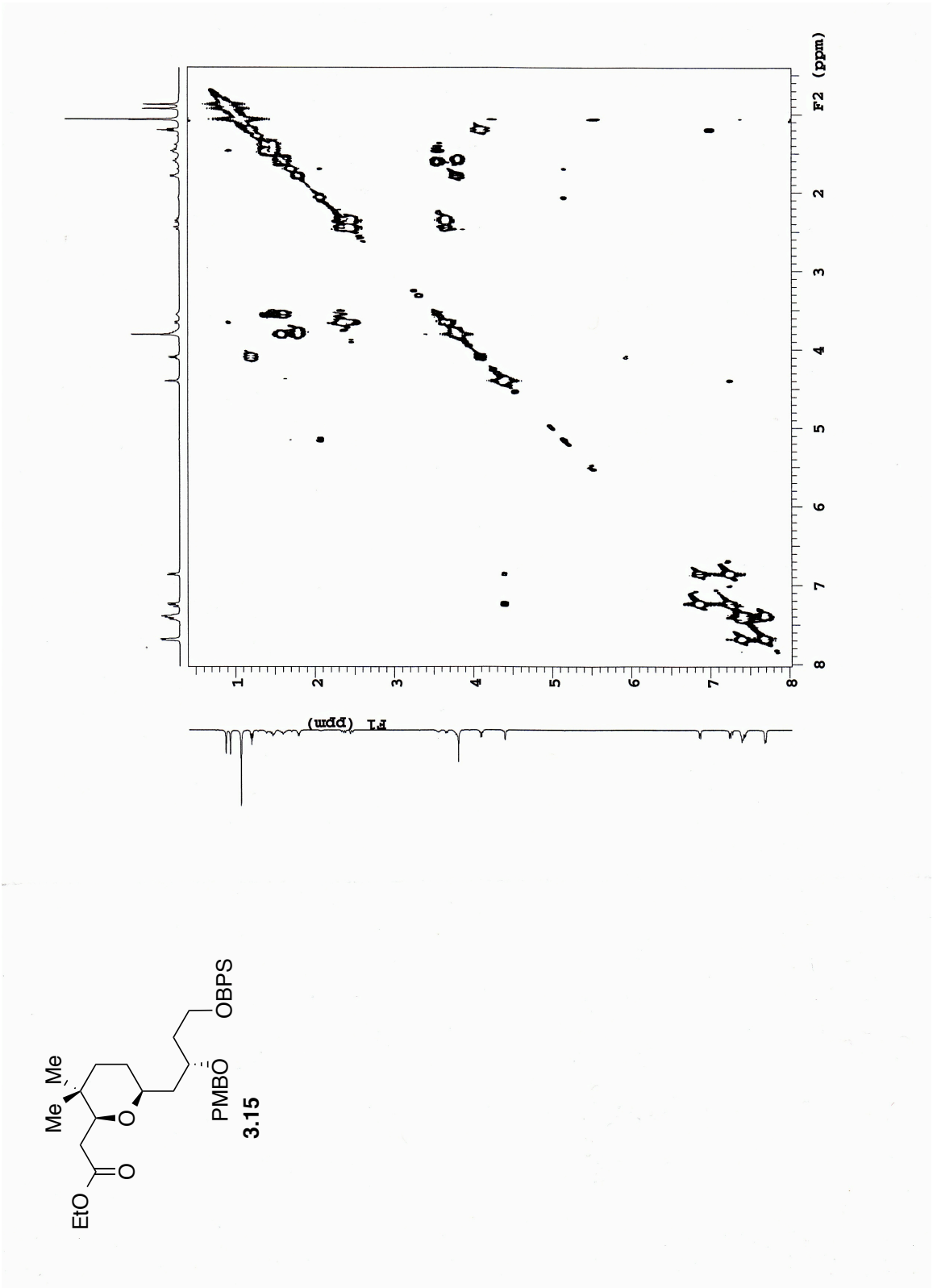

Me
Me
O
O
PMBO
OBPS
3.16
ppm
20
40
60
80
100
120
140
160
180
200


Me
Me
O
O
PMBO
OBPS
3.16
ppm
20
40
60
80
100
120
140
160
180
200

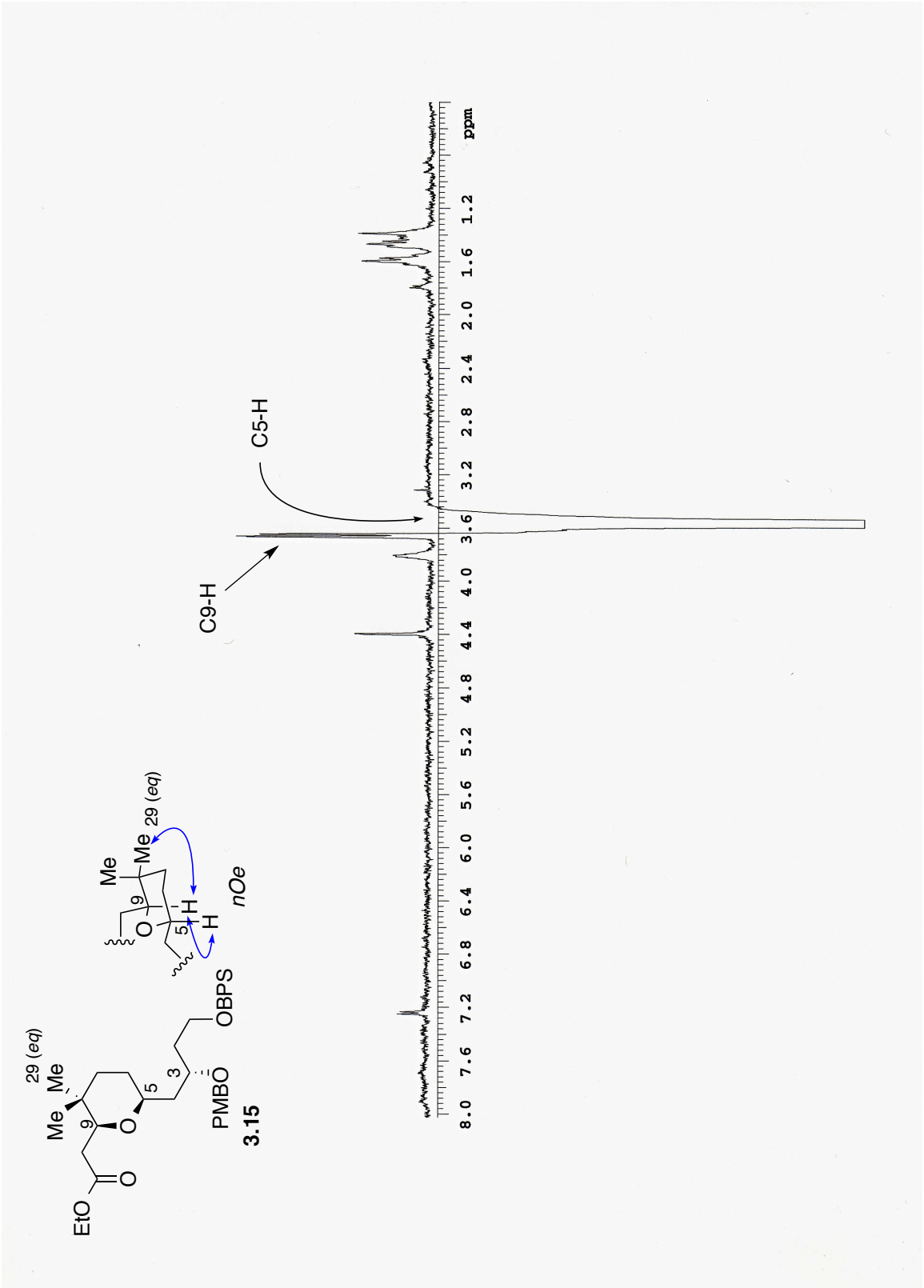

Me
Me
O
O
EtO
PMBO
OBPS
3.15
ppm
1
2
3
4
5
6
7
8
9

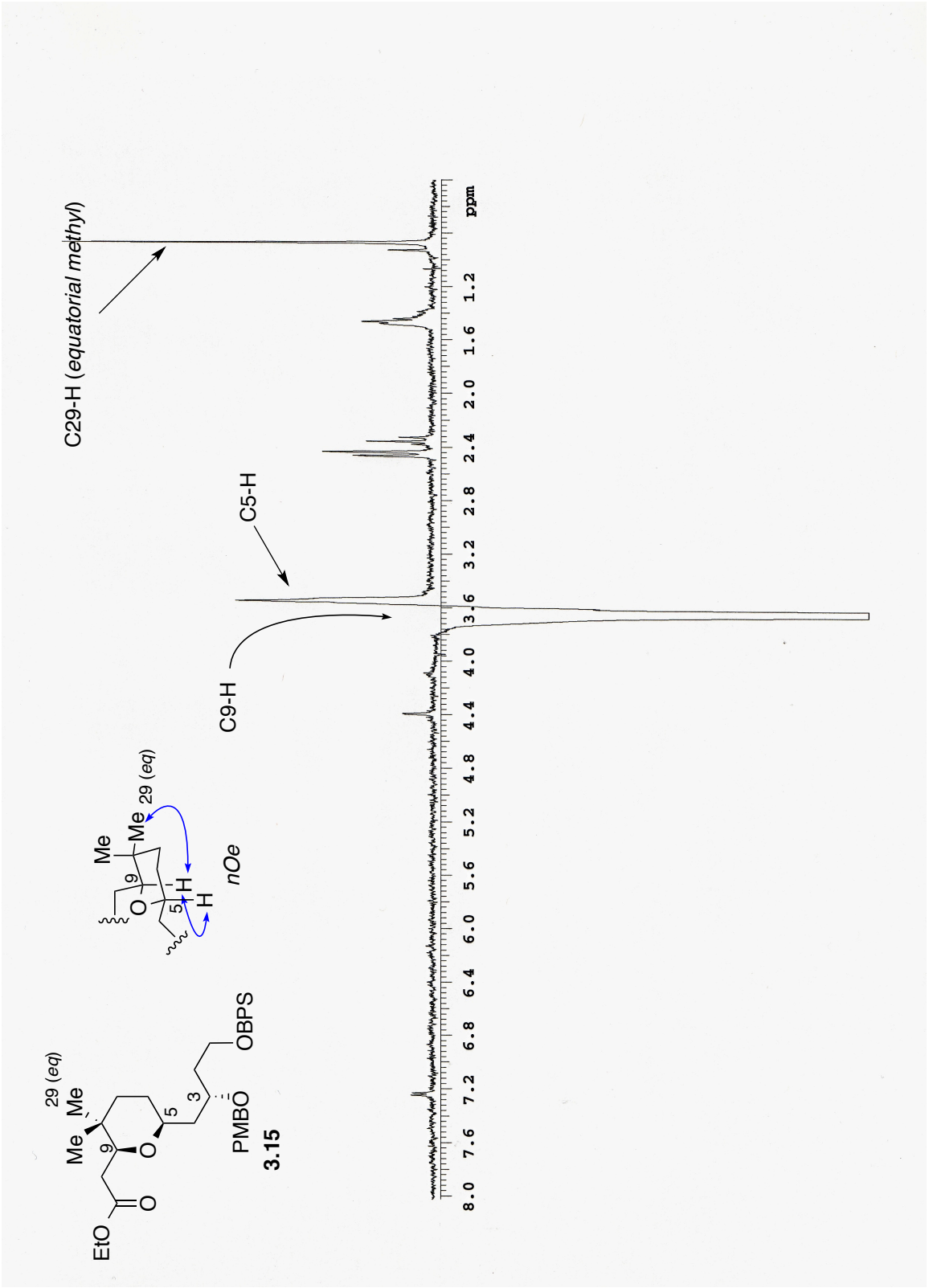

Me
Me
O
O
EtO
PMBO
OBPS
3.15
ppm
200
180
160
140
120
100
80
60
40
20

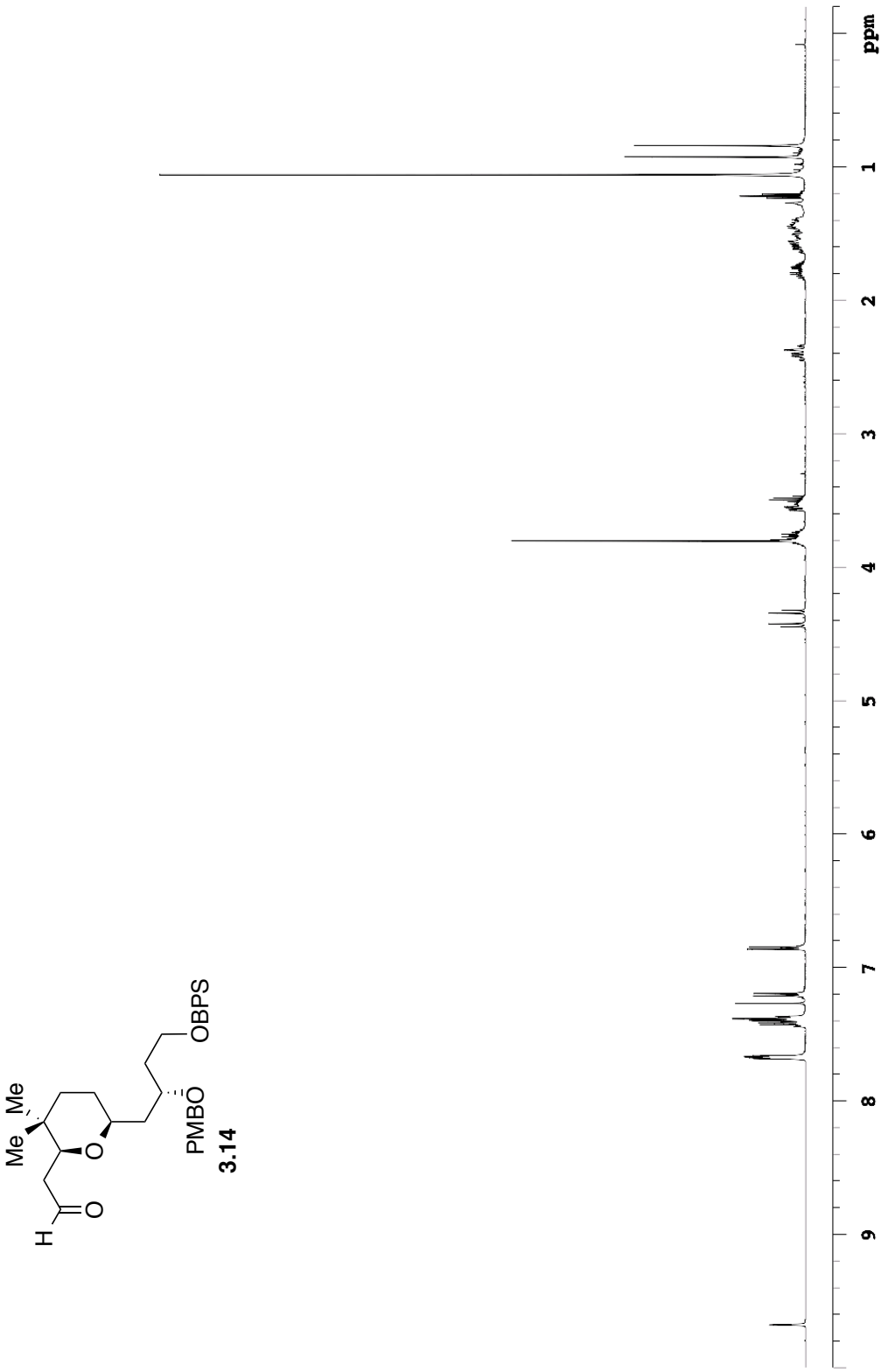

Me
Me
O
O
EtO
PMBO
OBPS
3.15
ppm
200
180
160
140
120
100
80
60
40
20

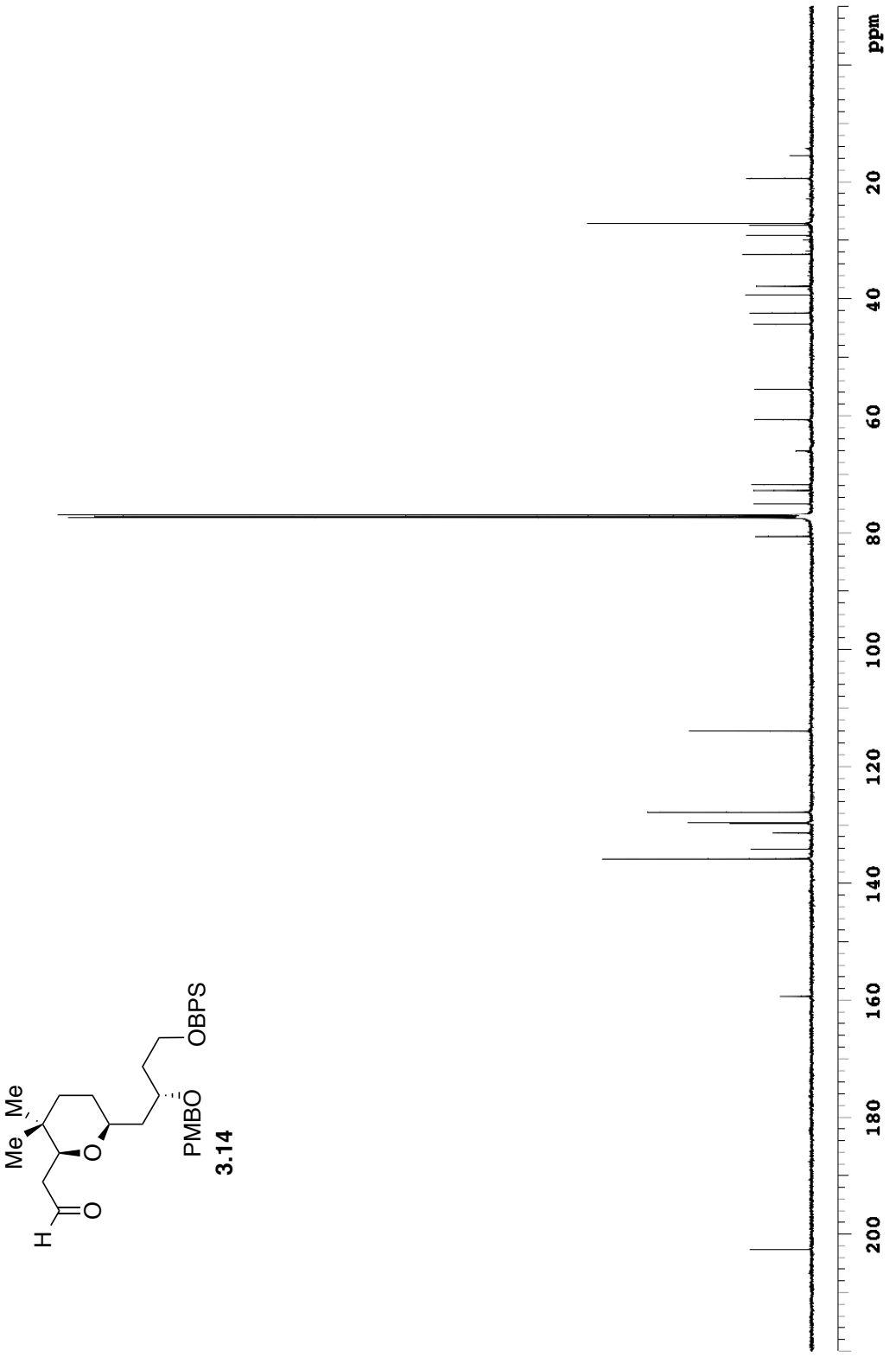


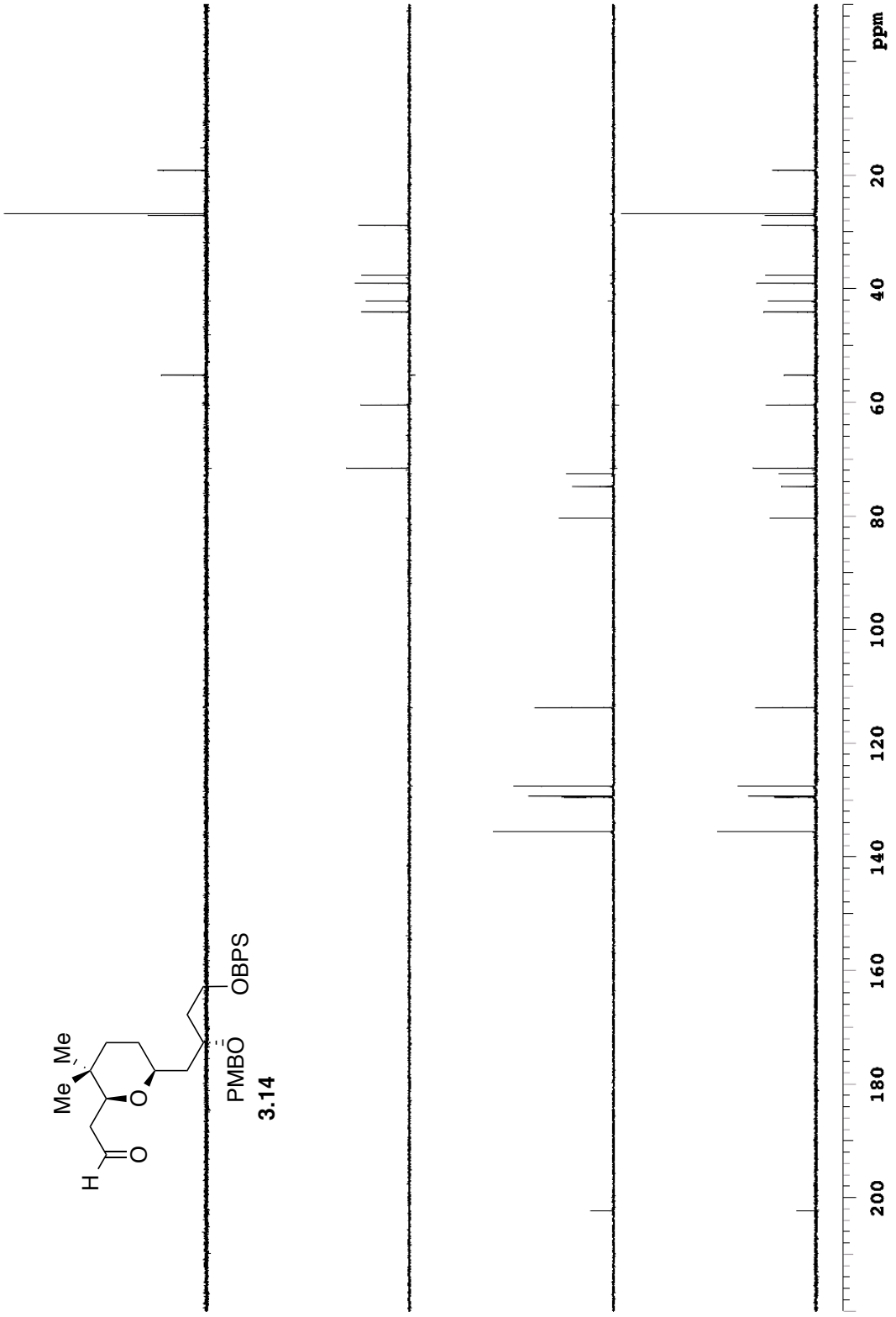
3.15

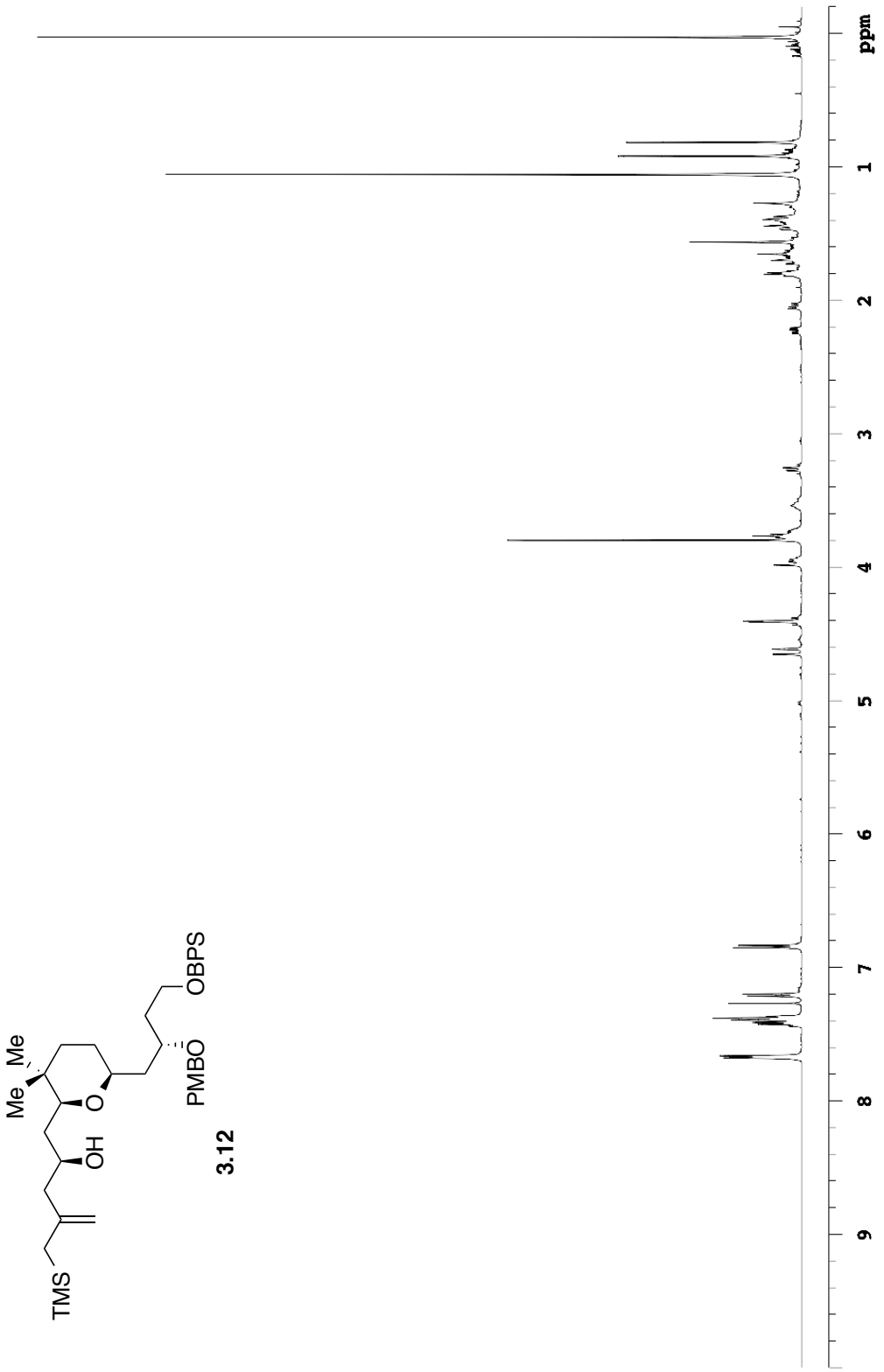

C9-H
C5-H
8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 ppm
29 (eq)
Me
Me
EtO
O
O
9
5
3
PMBO
OBPS
3.15
Me
Me
29 (eq)
O
9
5
H
H
nOe


C29-H (equatorial methyl)
C5-H
C9-H
ppm
8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2
29 (eq)
Me
Me
EtO
O
O
9
5
3
PMBO
OBPS
3.15
nOe
Me
Me
29 (eq)
H
H

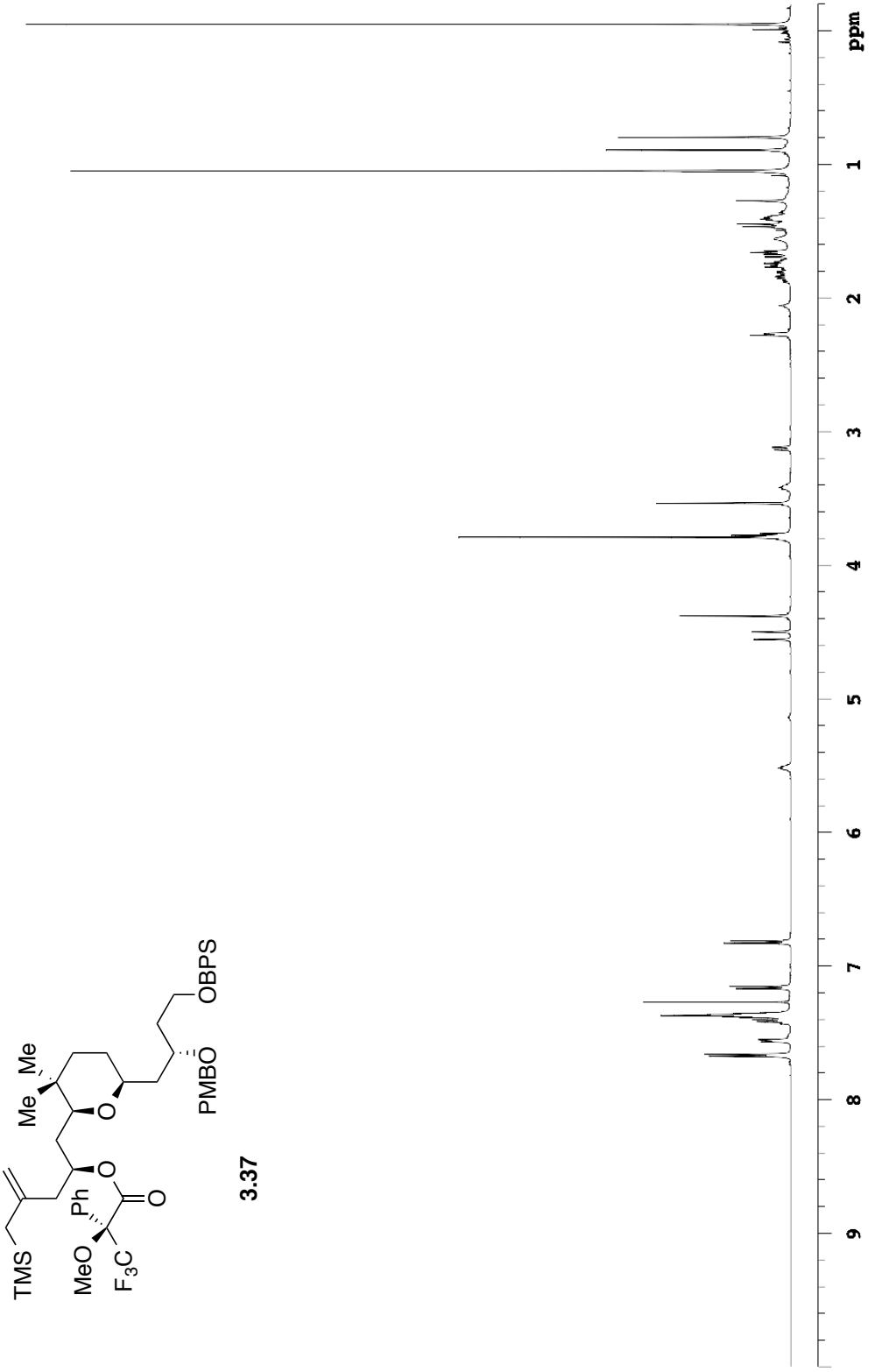

Me
Me
O
O
H
PMBO
OBPS
3.14
ppm
1
2
3
4
5
6
7
8
9


Me
Me
O
H
O
PMBO
OBPS
3.14
200
180
160
140
120
100
80
60
40
20
ppm

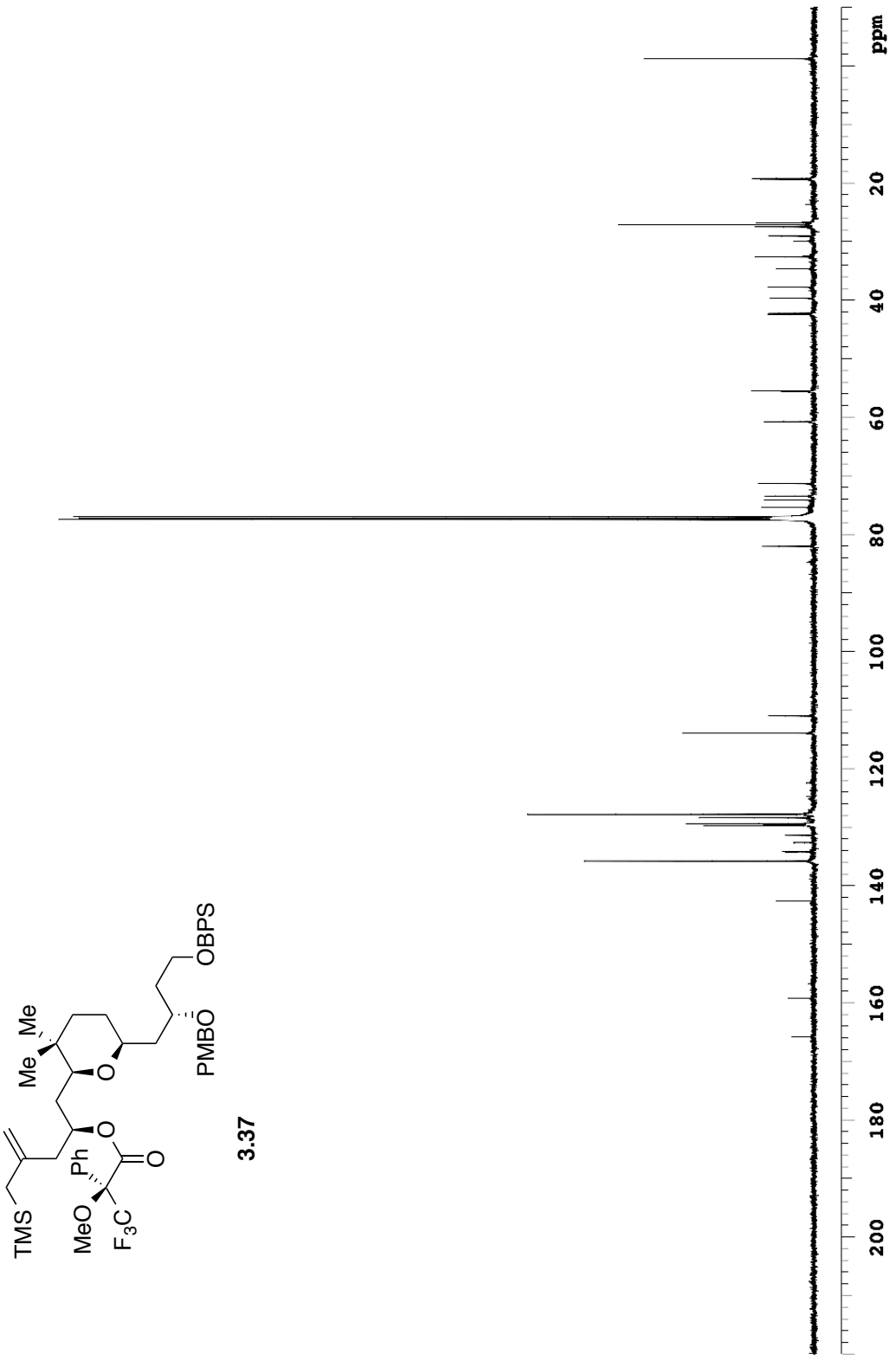

Me
Me
O
H
O
PMBO
OBPS
3.14
200
180
160
140
120
100
80
60
40
20
ppm

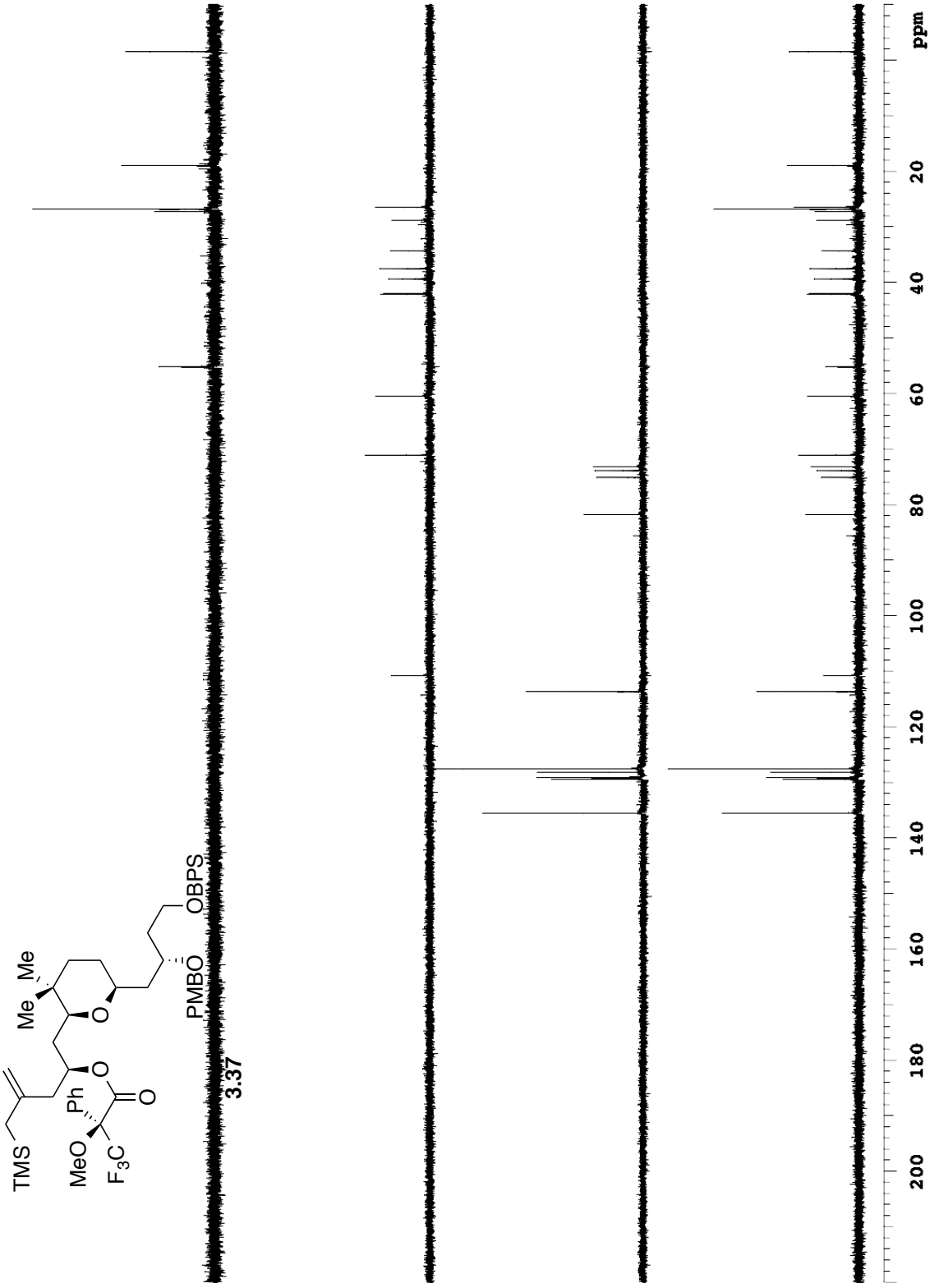


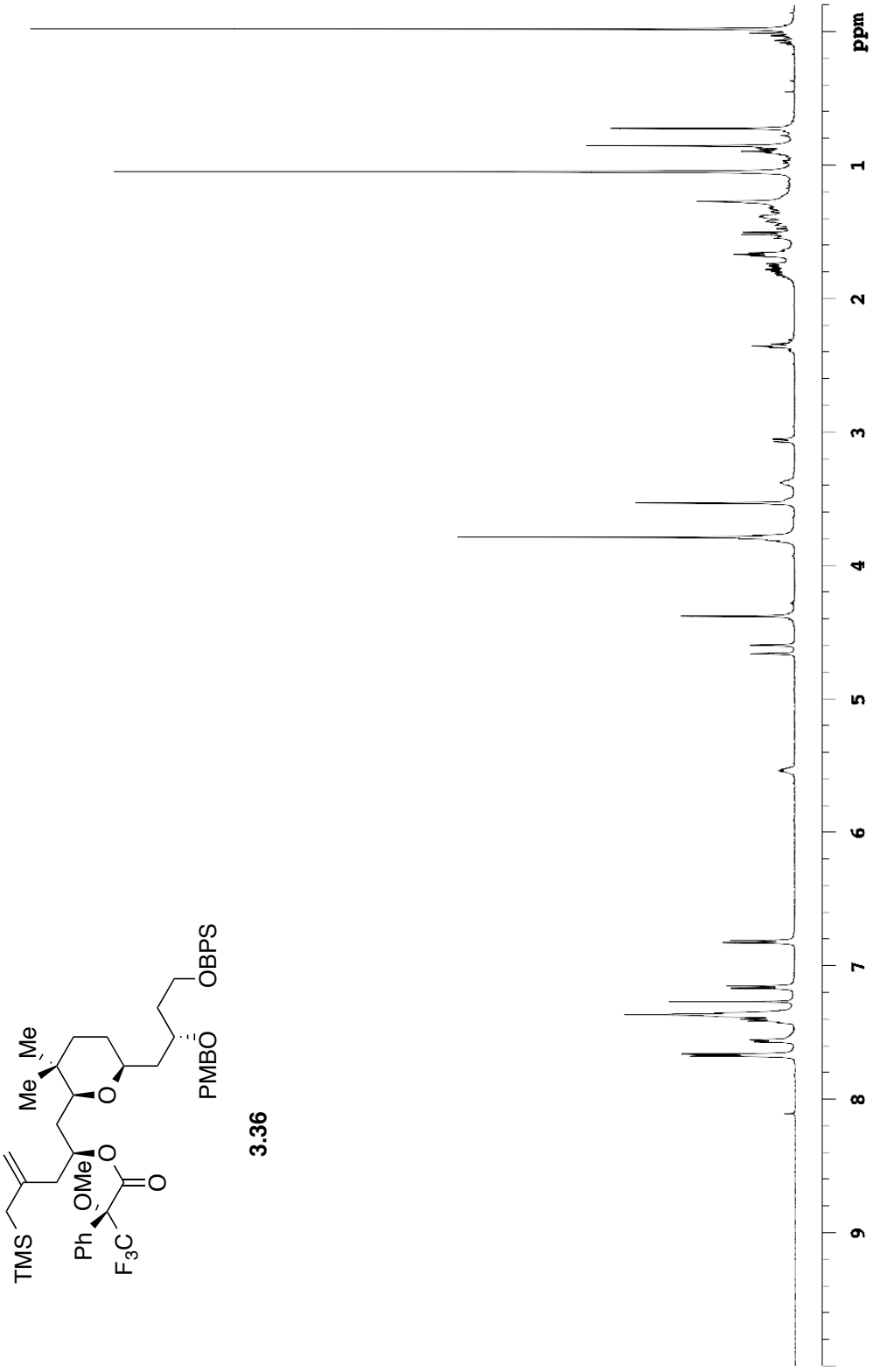
3.12



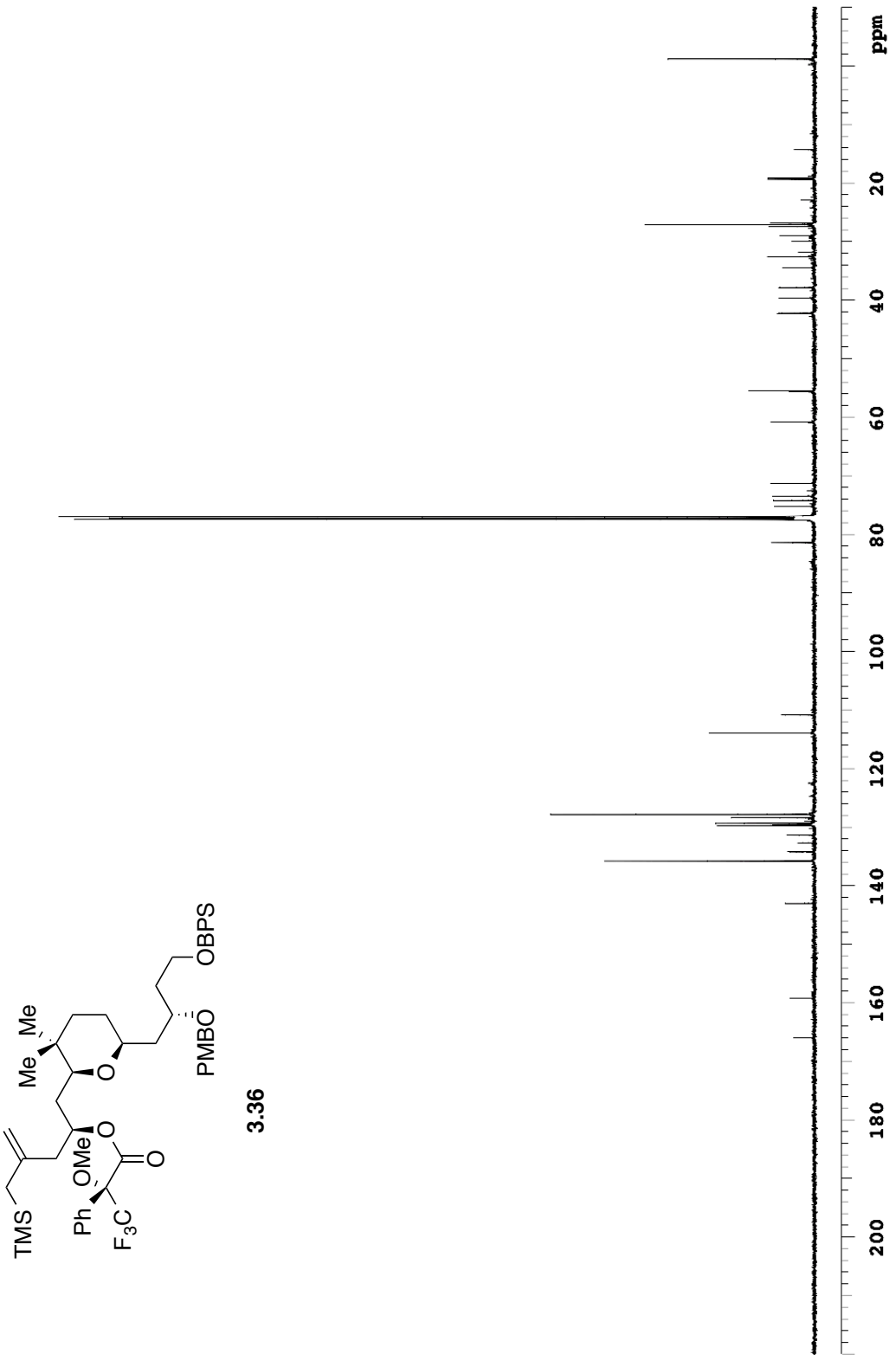
3.37


TMS
MeO
F3C
Ph
O
O
O
Me
Me
O
PMBO
OBPS
3.37
200
180
160
140
120
100
80
60
40
20
ppm

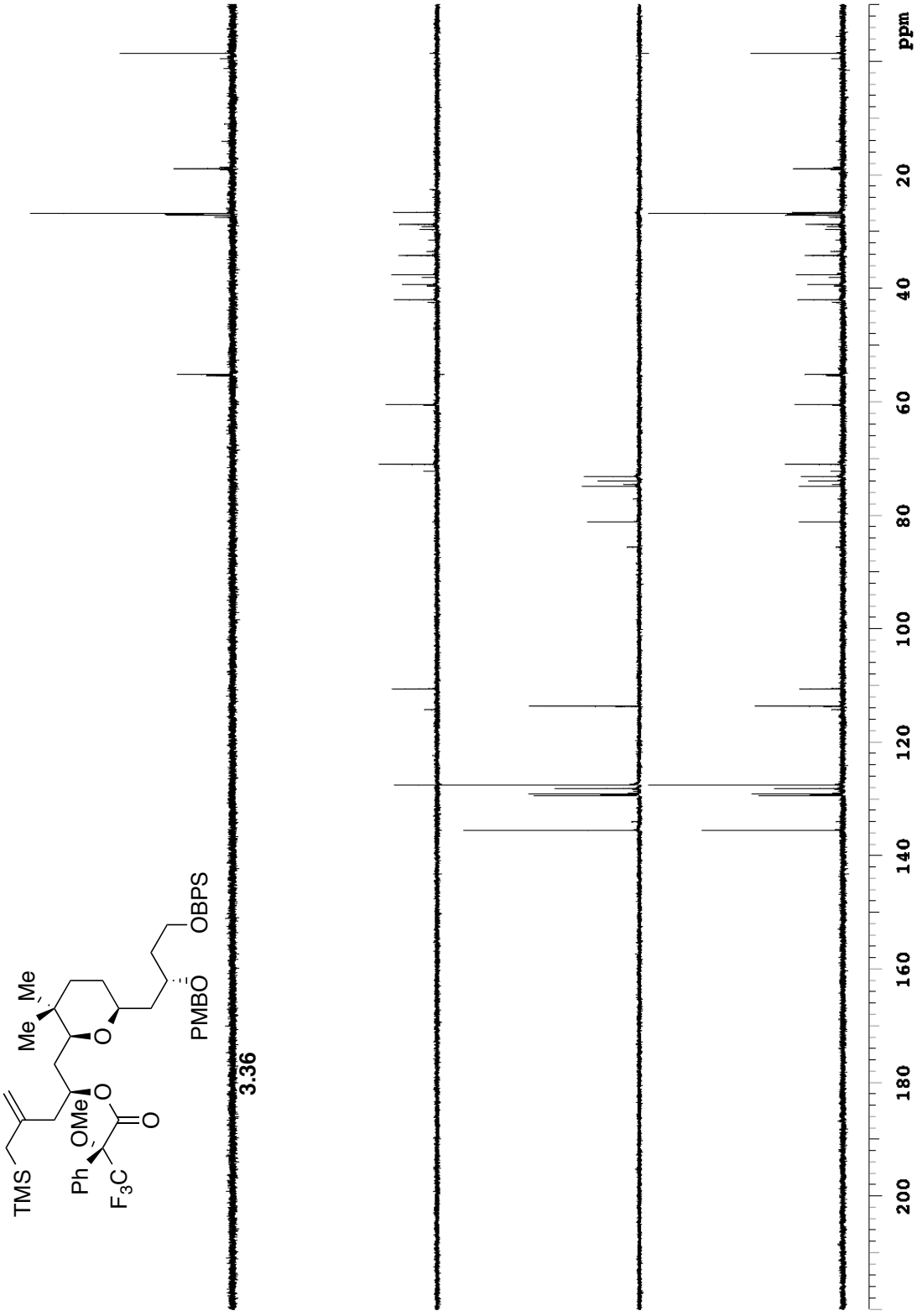

TMS
MeO
Ph
F3C
O
O
O
Me
Me
O
PMBO
OBPS
3.37
ppm
200
180
160
140
120
100
80
60
40
20

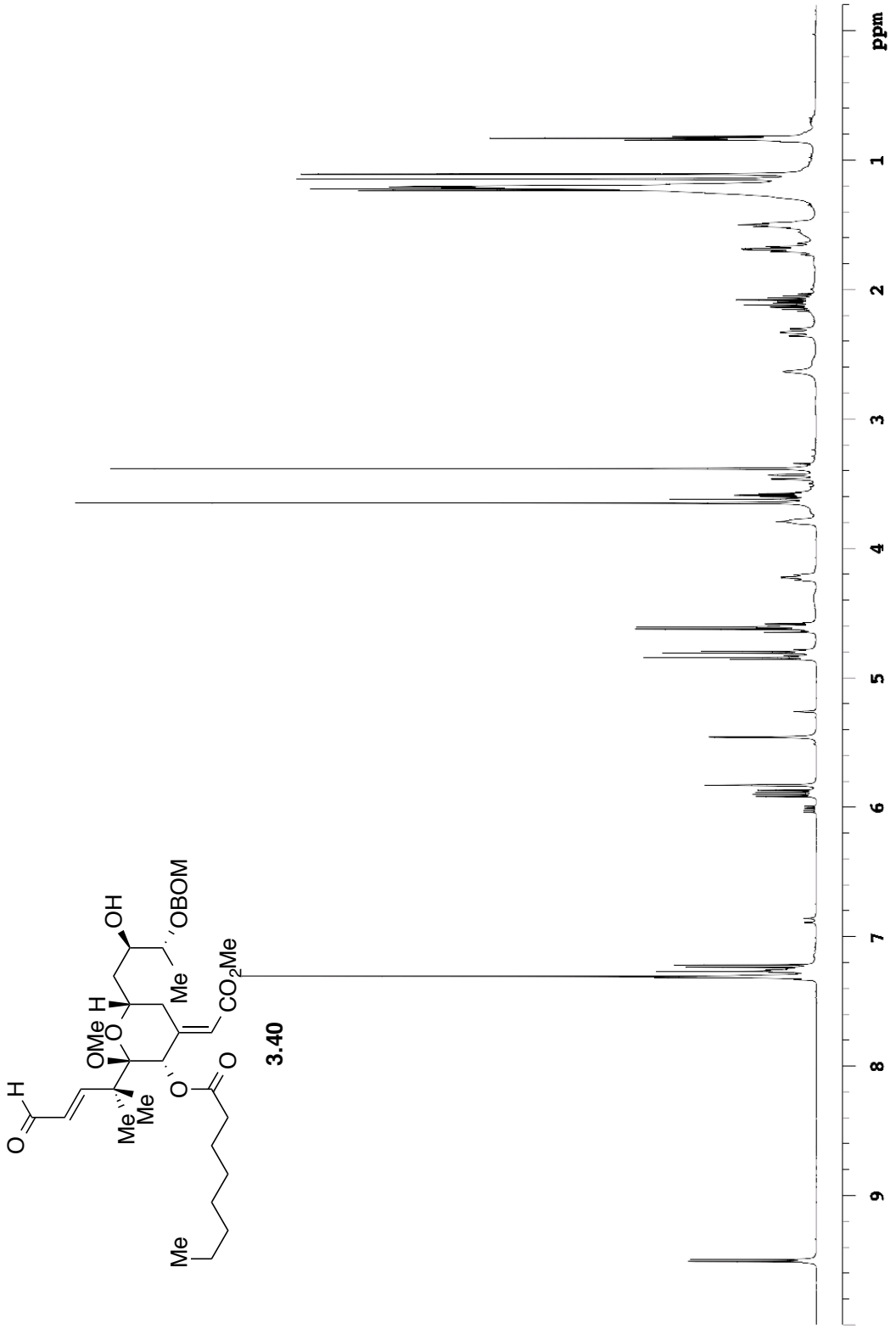


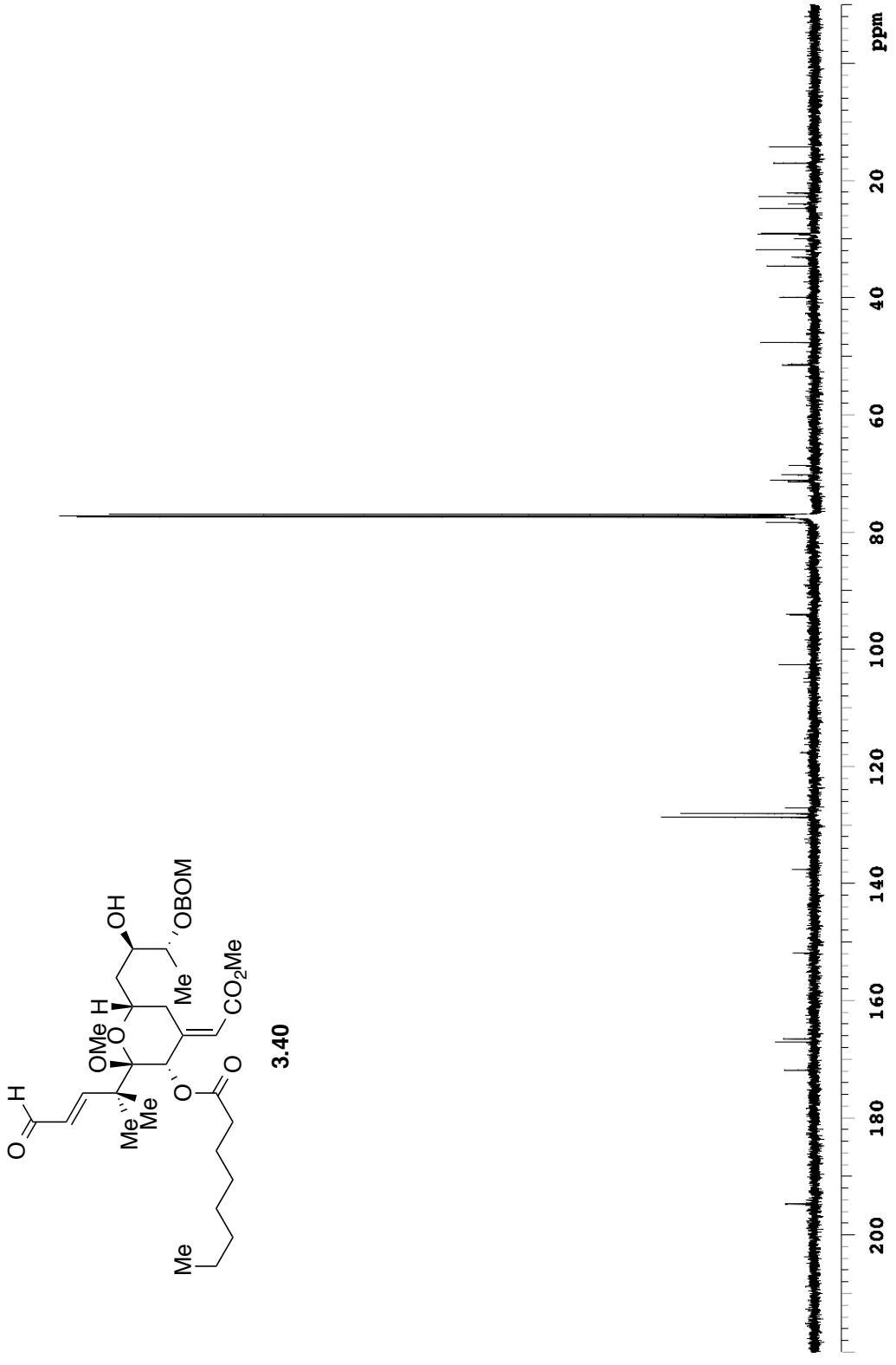
3.36

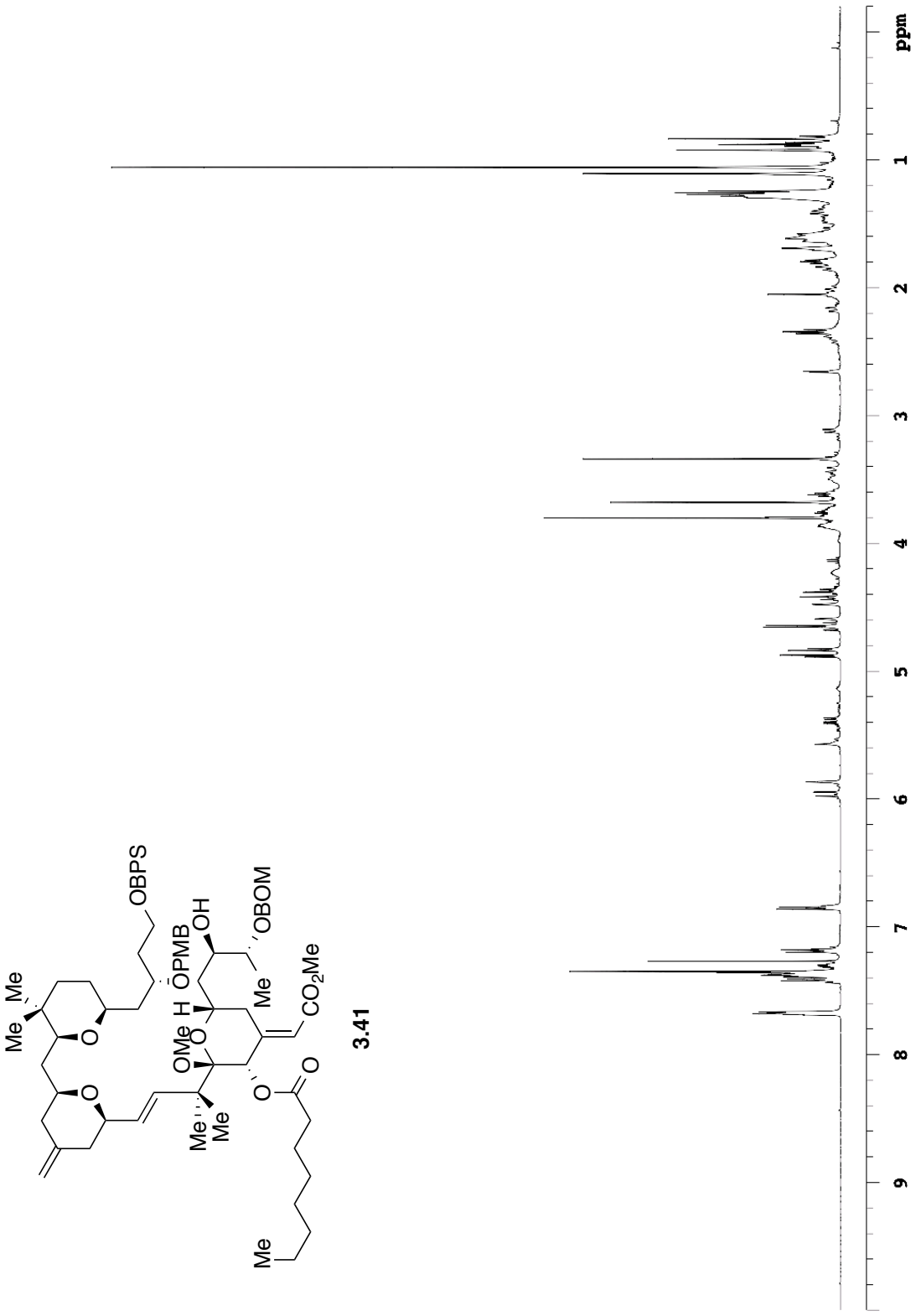

TMS
Ph
OMe
O
O
F3C
Me
Me
O
PMBO
OBPS
ppm
200
180
160
140
120
100
80
60
40
20

3.36

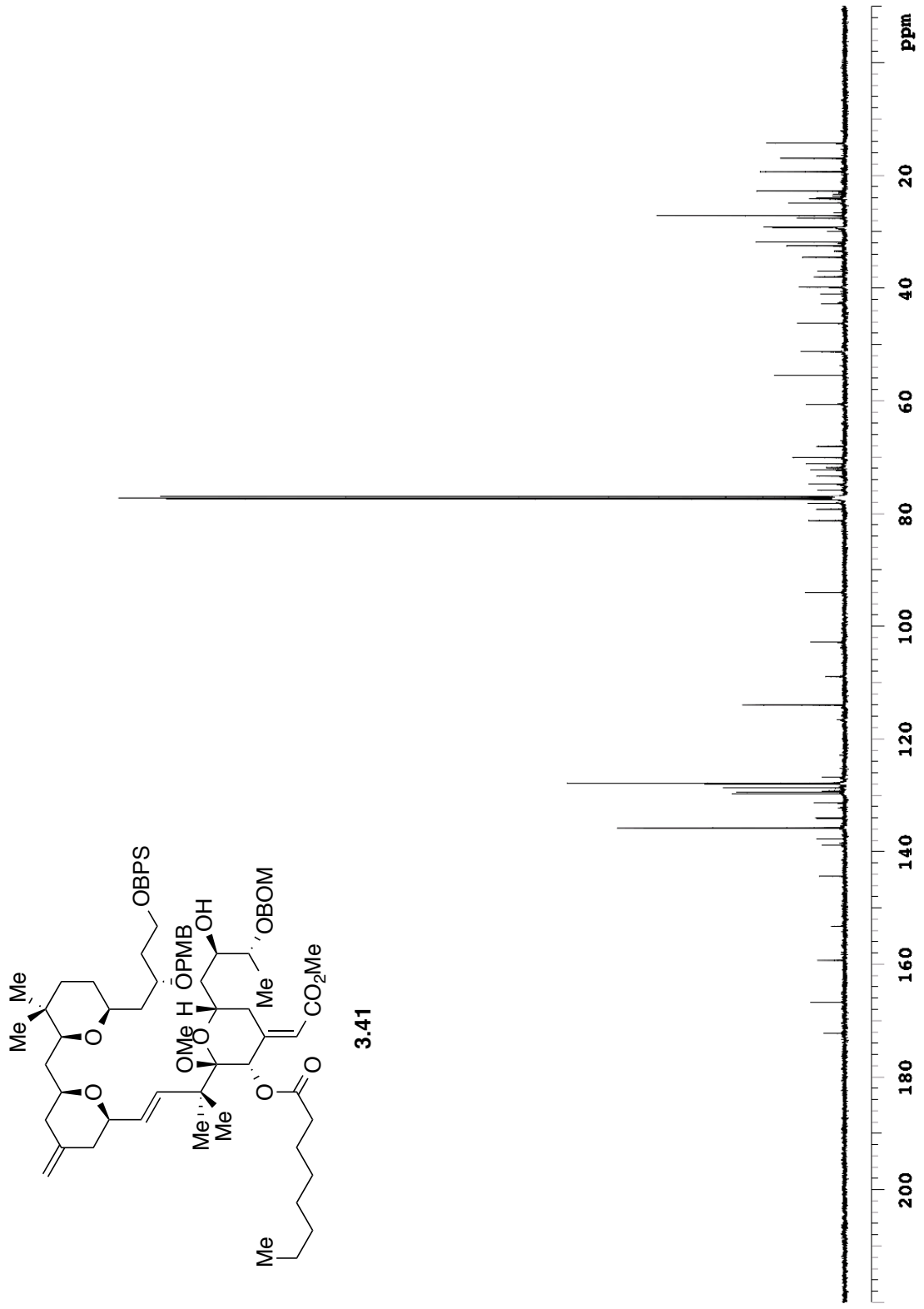

TMS
Ph
OMe
F3C
O
O
O
Me
Me
O
PMBO
OBPS
3.36
200
180
160
140
120
100
80
60
40
20
ppm

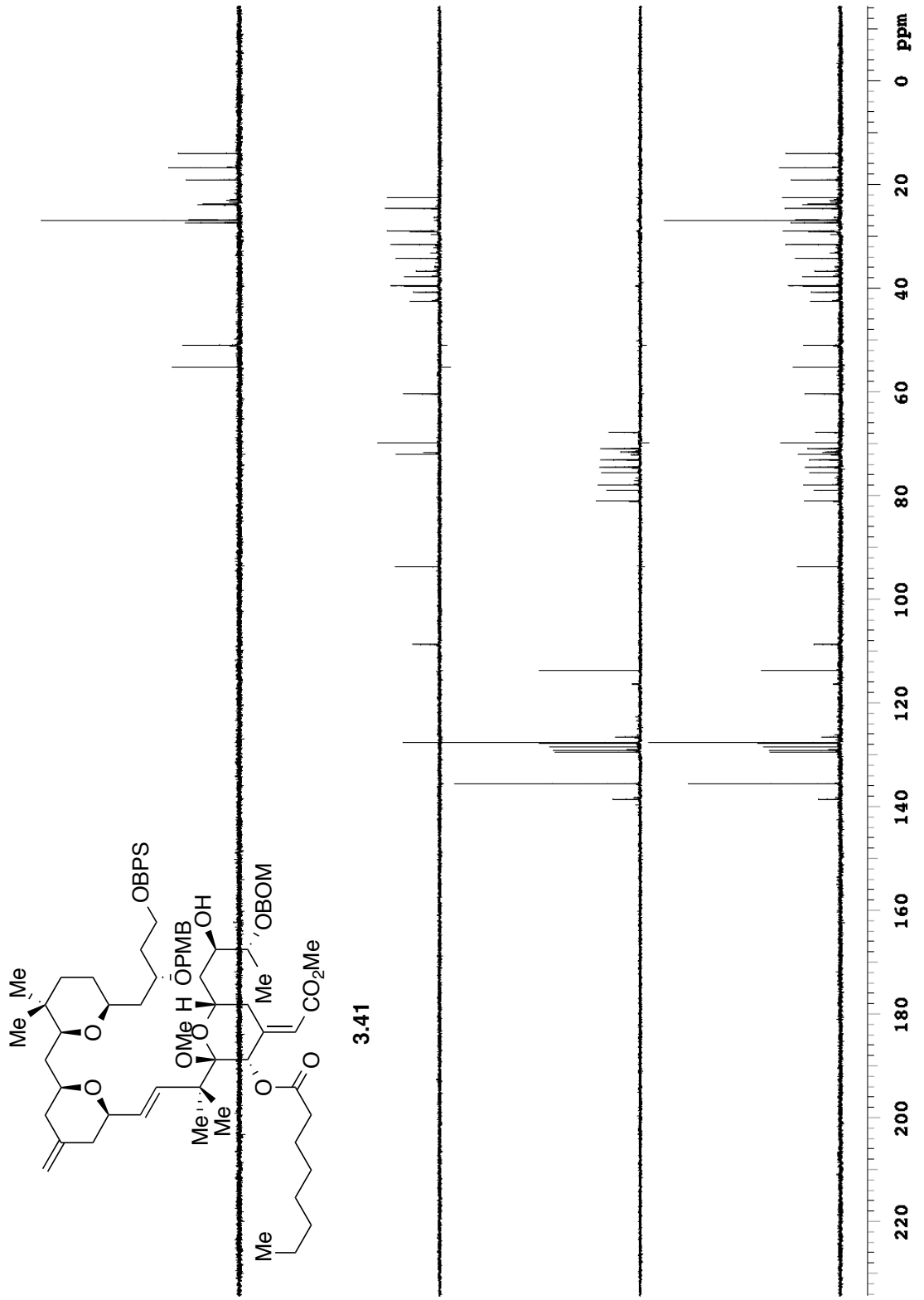

ppm
1
2
3
4
5
6
7
8
9
3.40
OMe
H
O
OH
OBOM
Me
CO2Me
Me
Me
O
O
O
H
Me

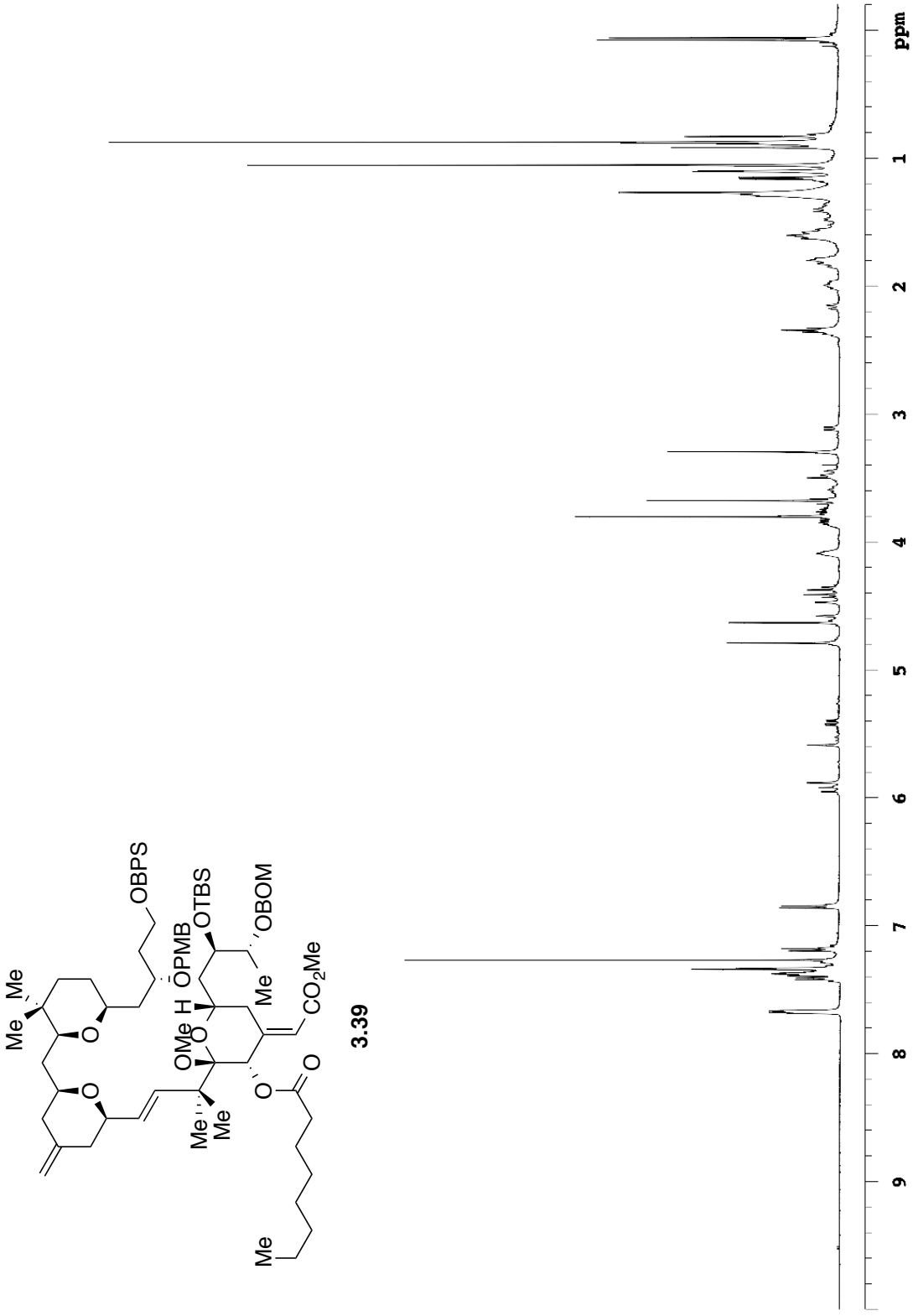

OH
OBOM
CO2Me
OMe
H
O
Me
Me
O
O
H
O
Me
3.40
ppm
200
180
160
140
120
100
80
60
40
20


OBPS
OPMB
OH
OBOM
Me
Me
OMe
H
O
Me
Me
Me
CO2Me
O
O
Me
ppm
1
2
3
4
5
6
7
8
9

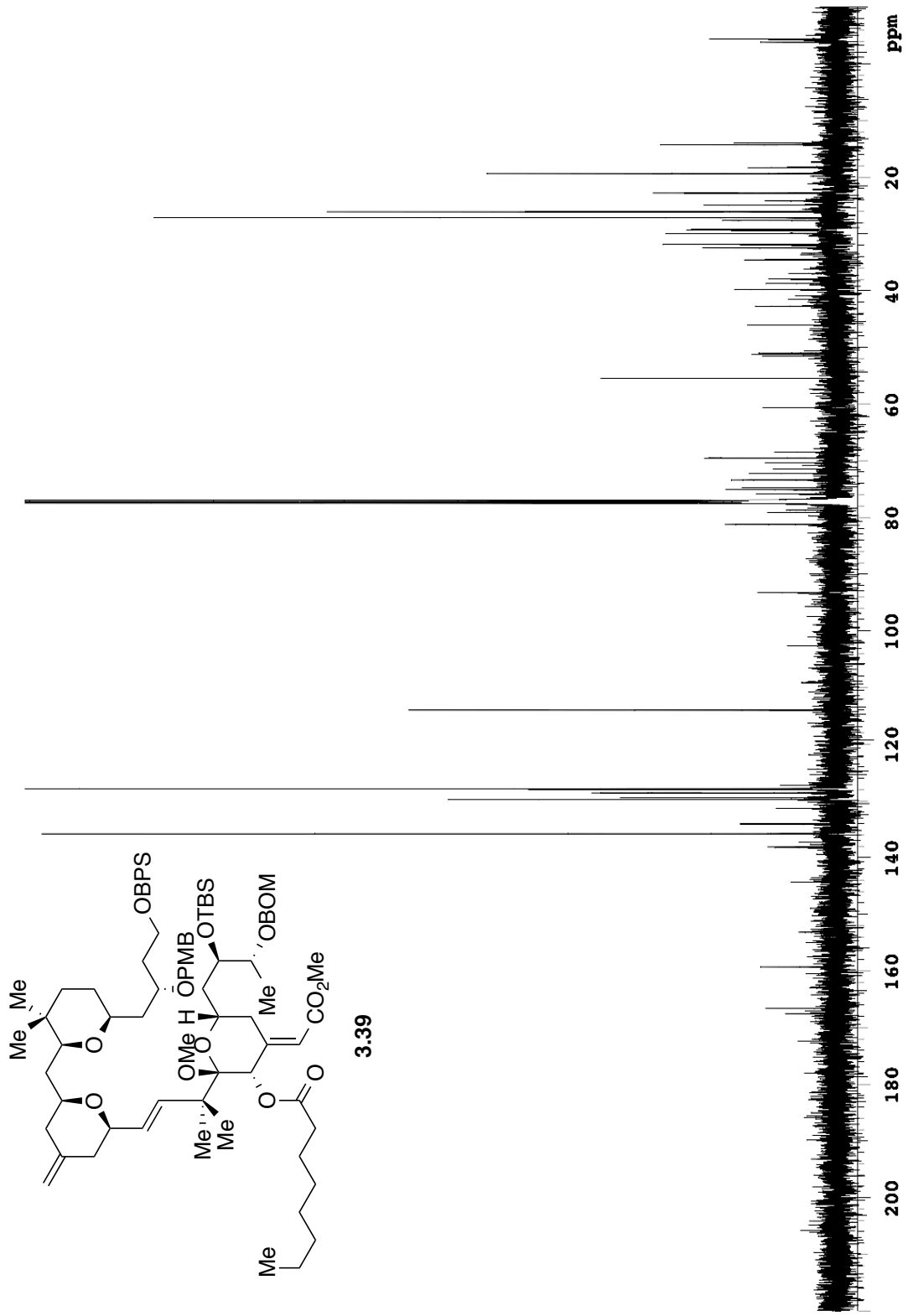
3.41


OBPS
OMe H OPMB
OH
OBOM
Me
Me
Me
Me
Me
CO2Me
O
O
O
O
O
O
Me
Me
Me
3.41
ppm
200
180
160
140
120
100
80
60
40
20

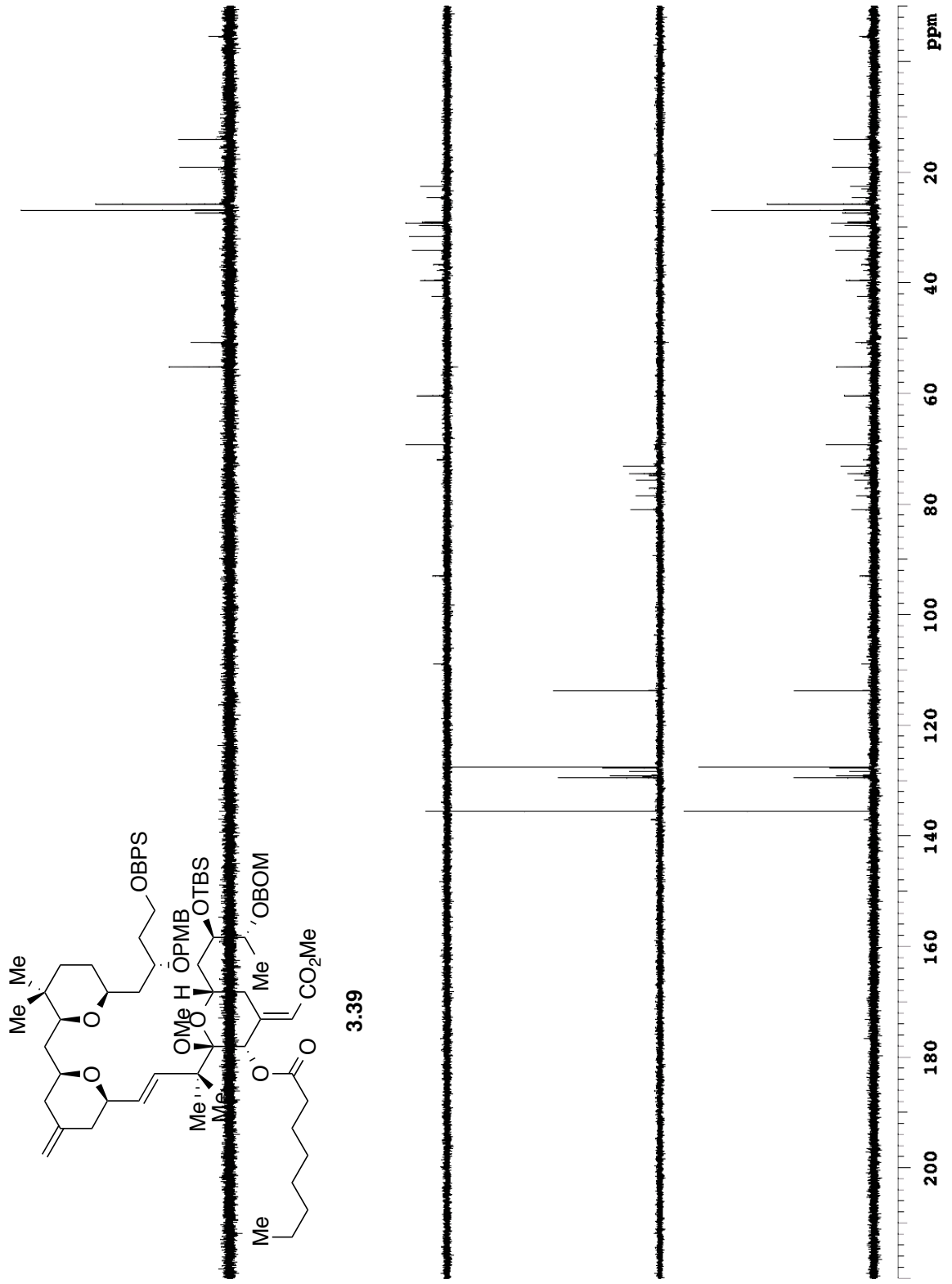

Me
Me
OBPS
OMe
H
OPMB
OH
Me
Me
Me
OBOM
CO2Me
O
O
O
O
O
O
Me
3.41
0
ppm
20
40
60
80
100
120
140
160
180
200
220


OBPS
OTBS
OPMB
OBOM
OMe
H
Me
Me
Me
Me
Me
Me
CO2Me
O
O
O
O
O
O
ppm
1
2
3
4
5
6
7
8
9

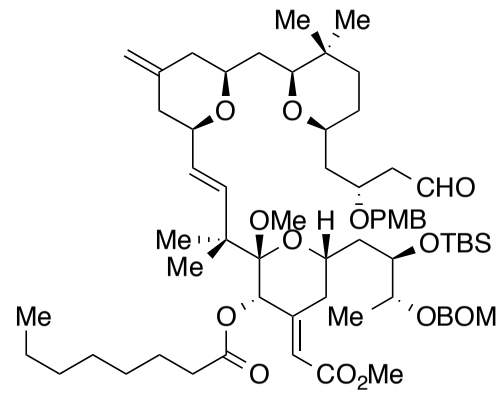
3.39


OBPS
OPMB
OTBS
OBOM
OMe
H
Me
Me
Me
Me
Me
Me
CO_2Me
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

3.39

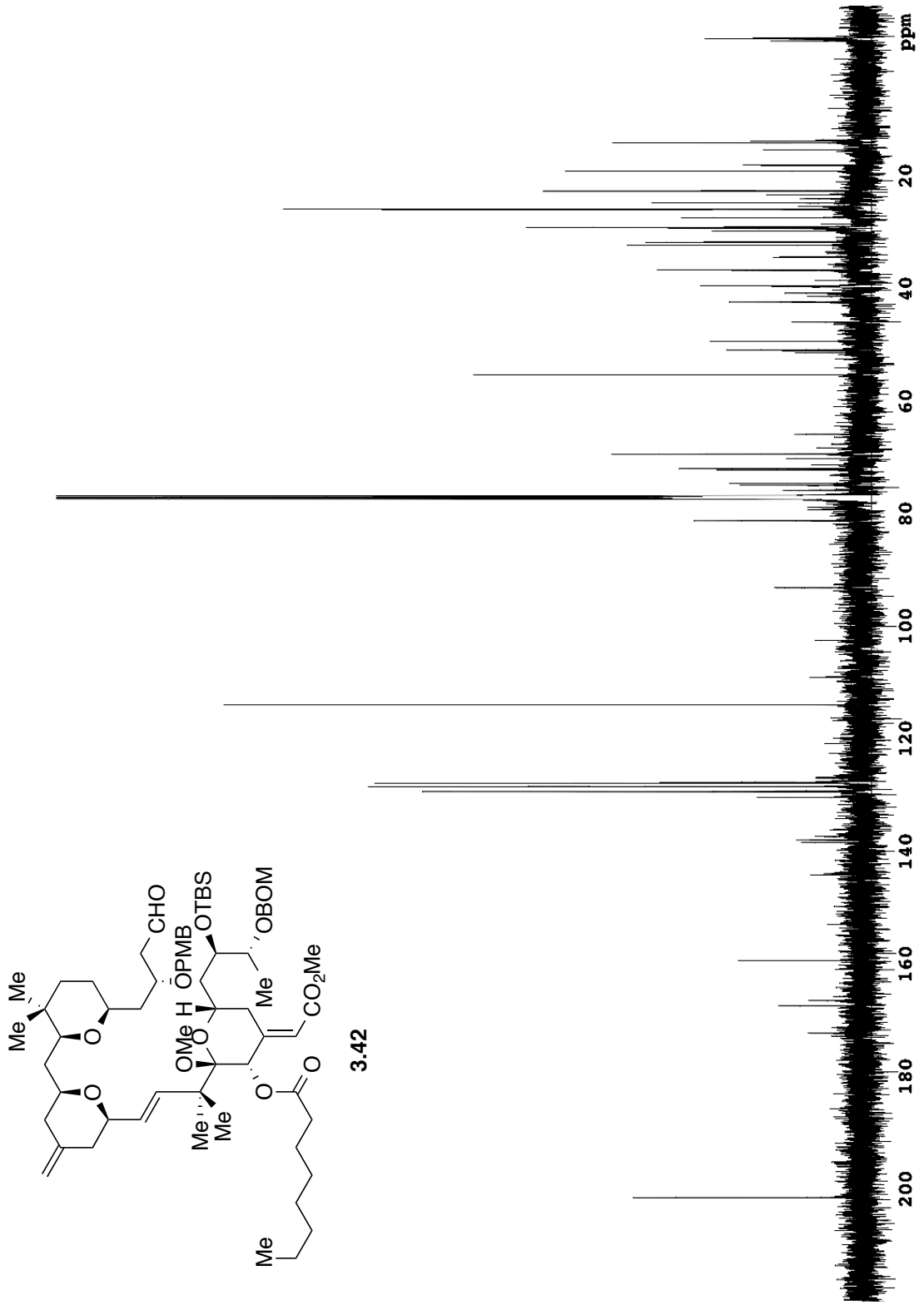

OBPS
OTBS
OPMB
OBOM
Me
Me
OMe
H
Me
Me
Me
Me
CO_2Me
O
O
O
O
O
O
ppm
200
180
160
140
120
100
80
60
40
20

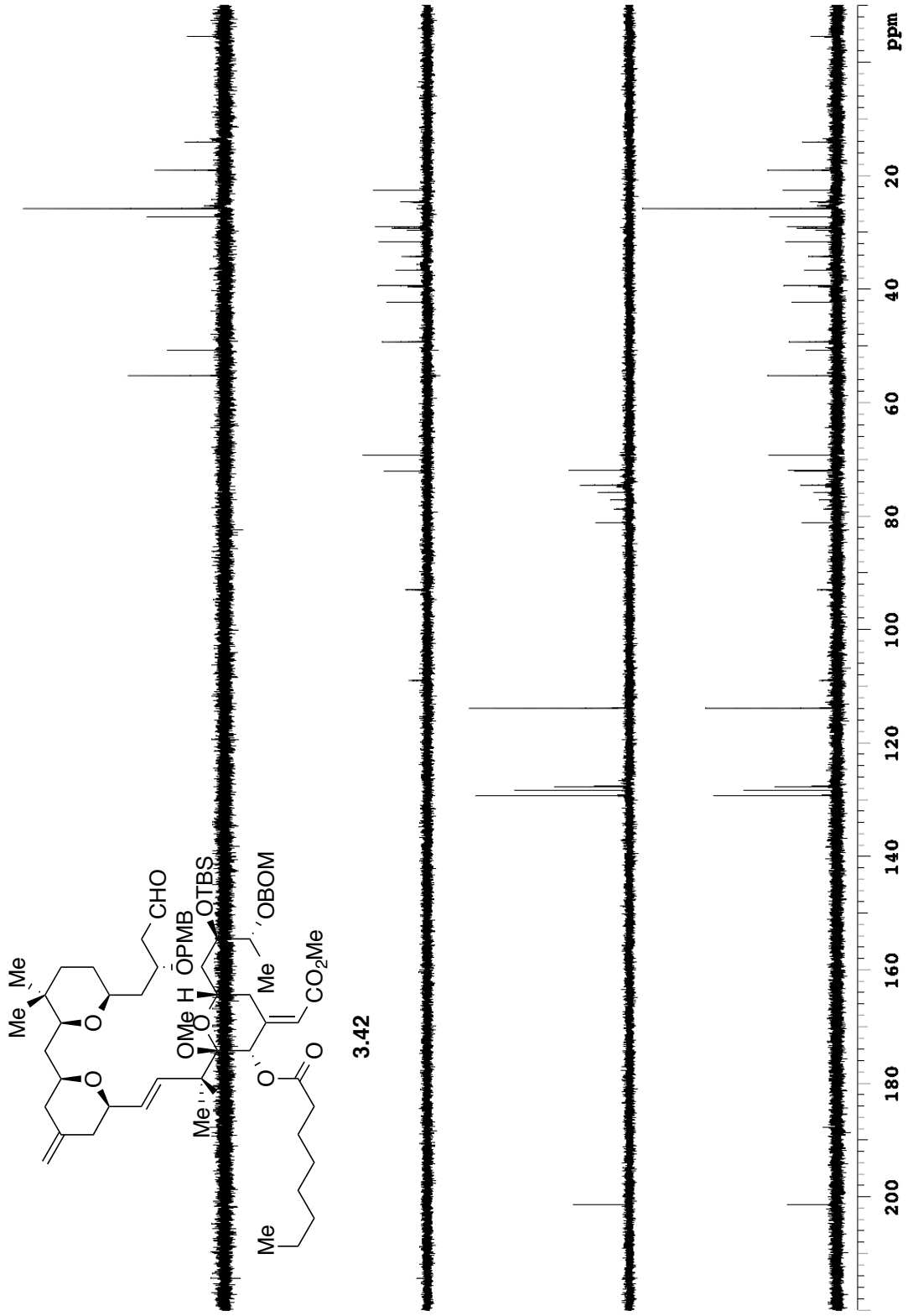
3.39

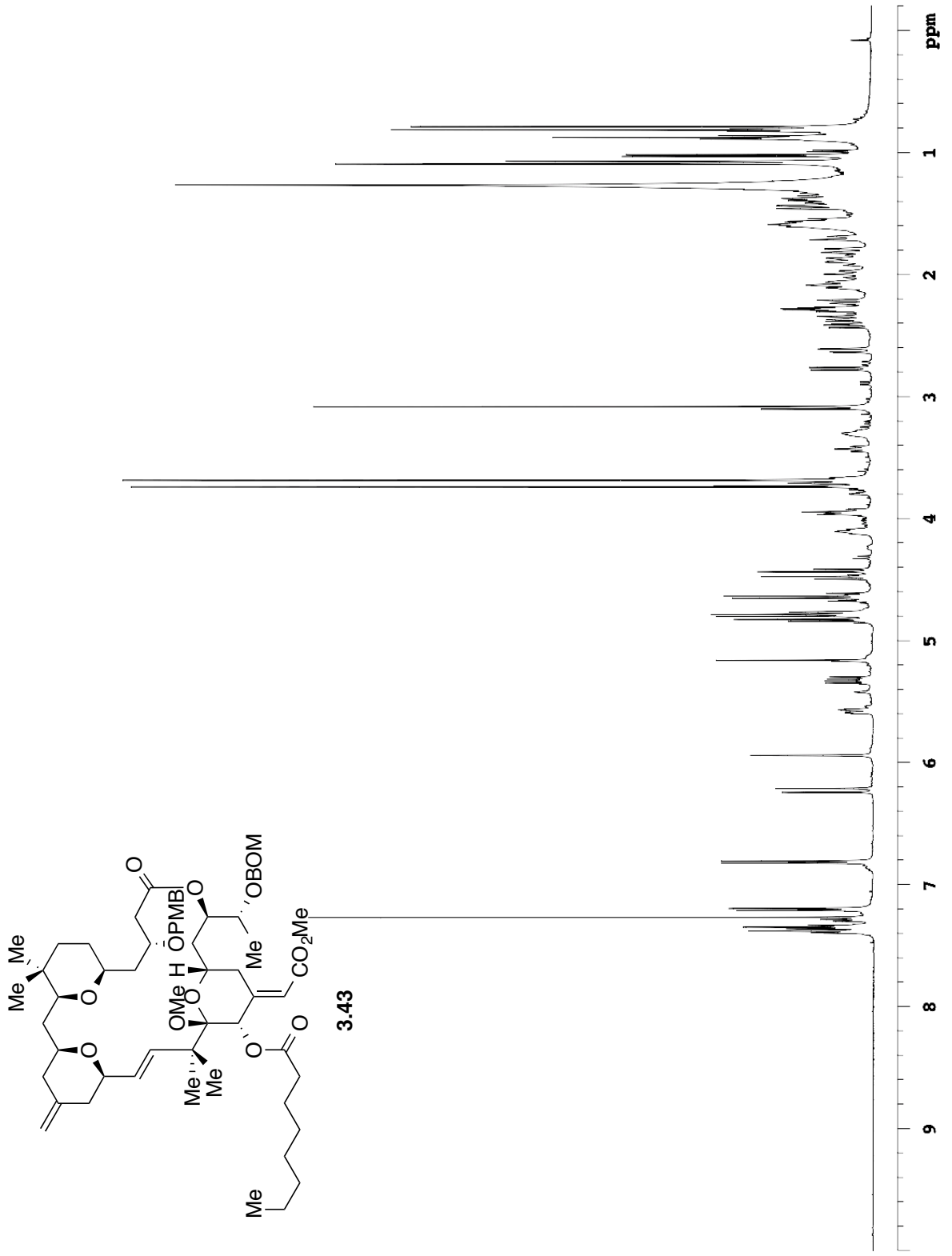

Me
Me
O
O
CHO
OMe
H
OPMB
Me
Me
O
OTBS
Me
O
Me
OBOM
O
O
CO2Me

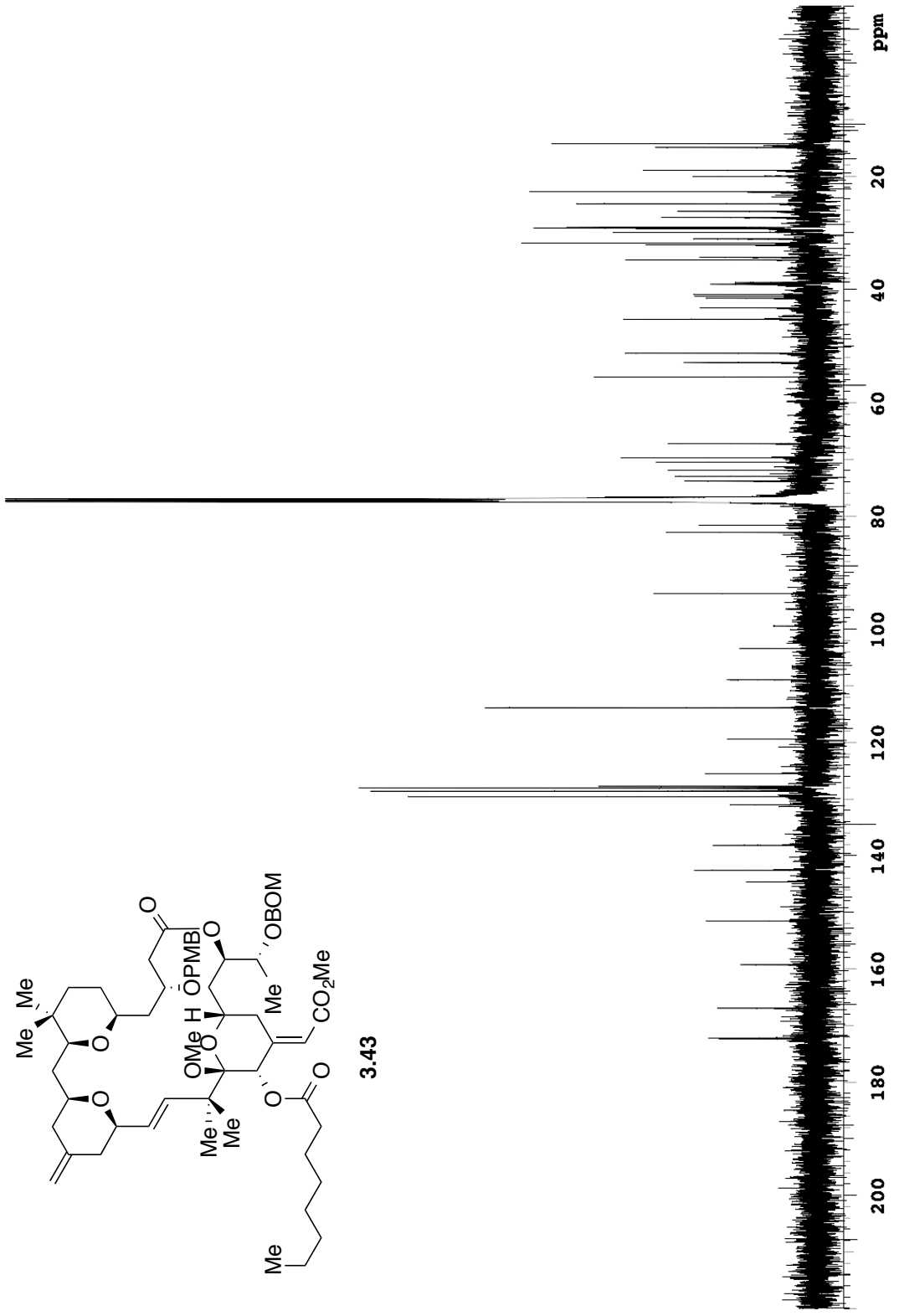
3.42

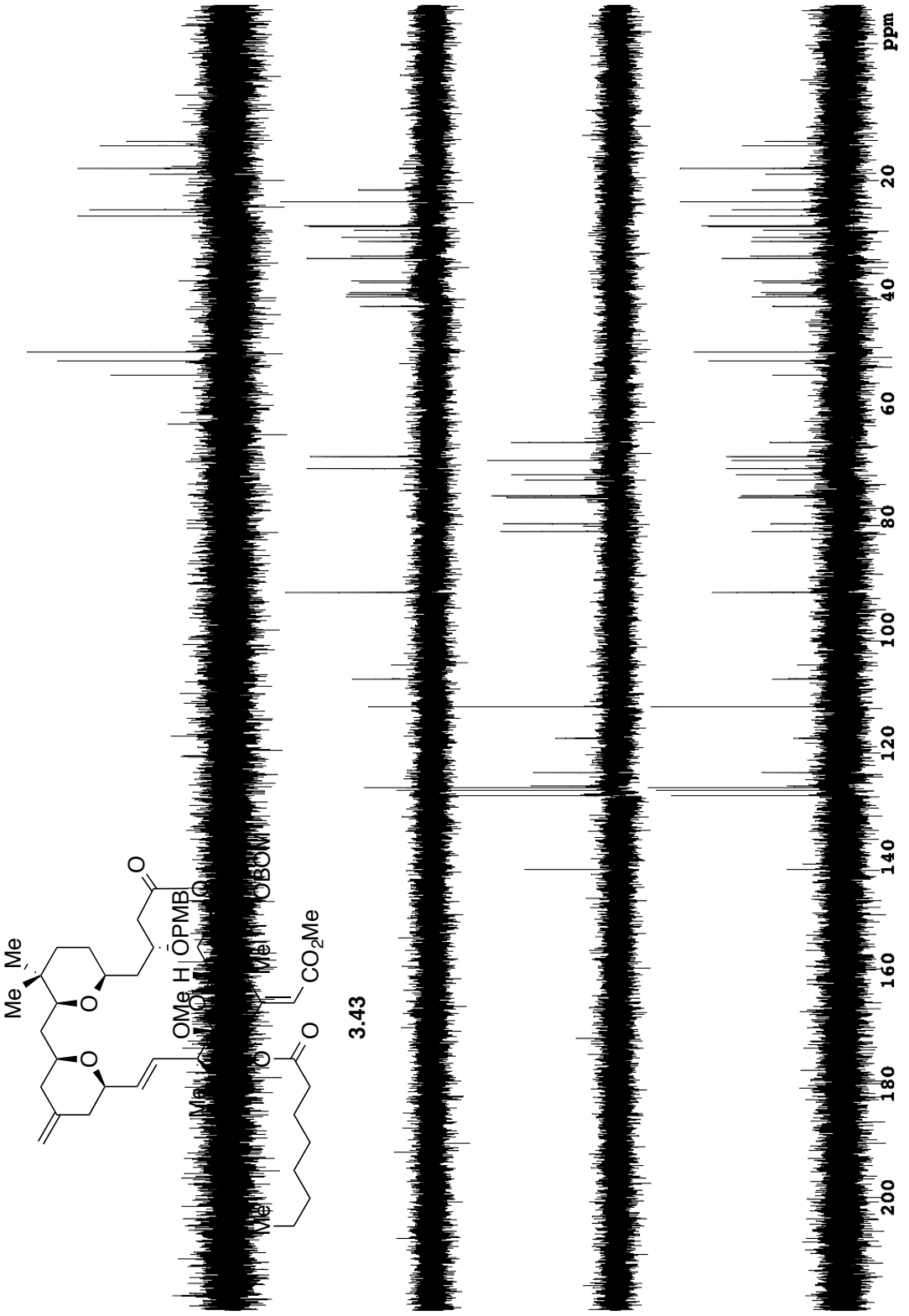
9
8
7
6
5
4
3
2
1
ppm

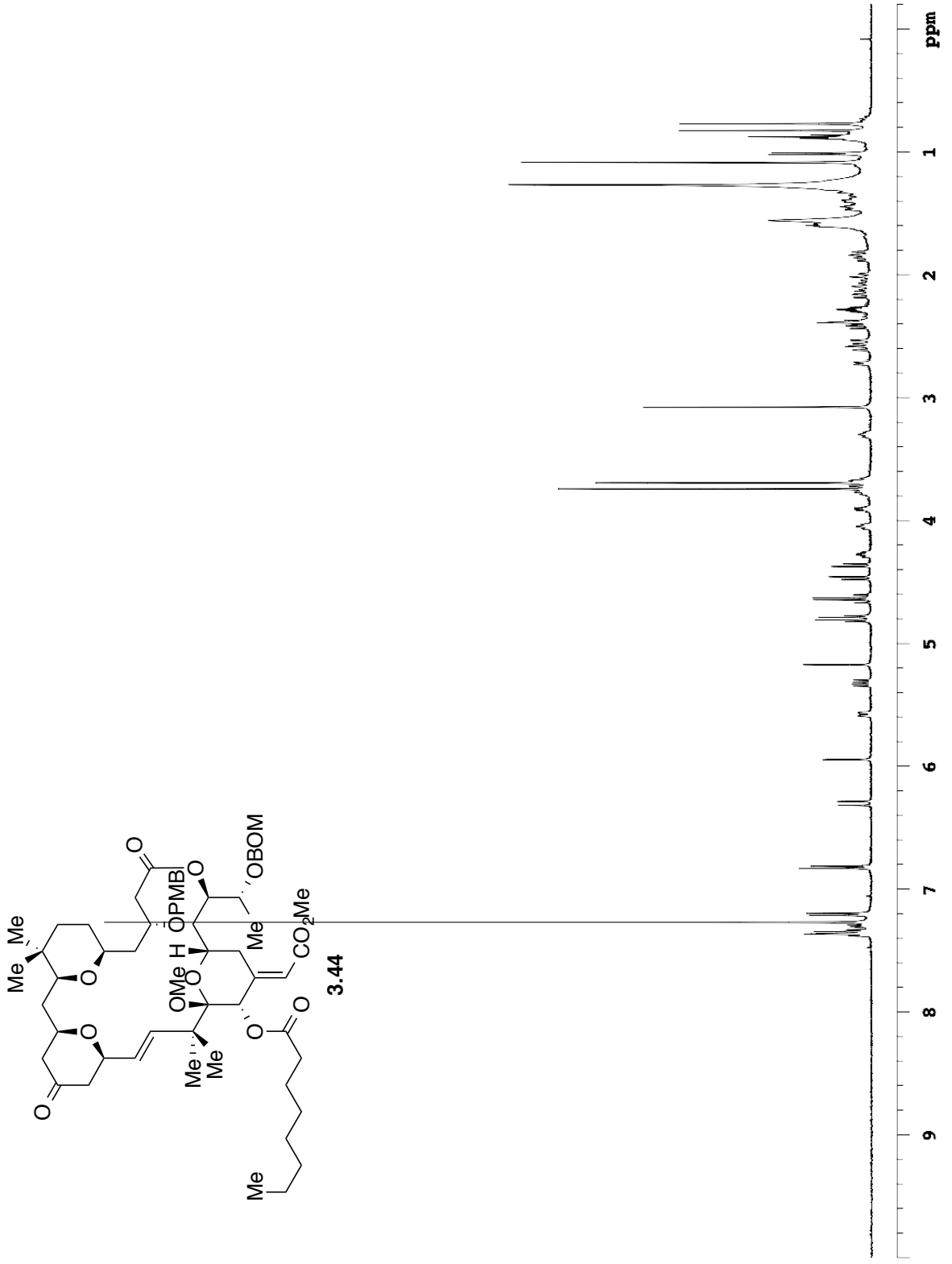

Me
Me
O
O
CHO
OPMB
H
OMe
O
OTBS
Me
Me
O
Me
OBOM
CO2Me
O
O
Me
3.42
ppm
200
180
160
140
120
100
80
60
40
20

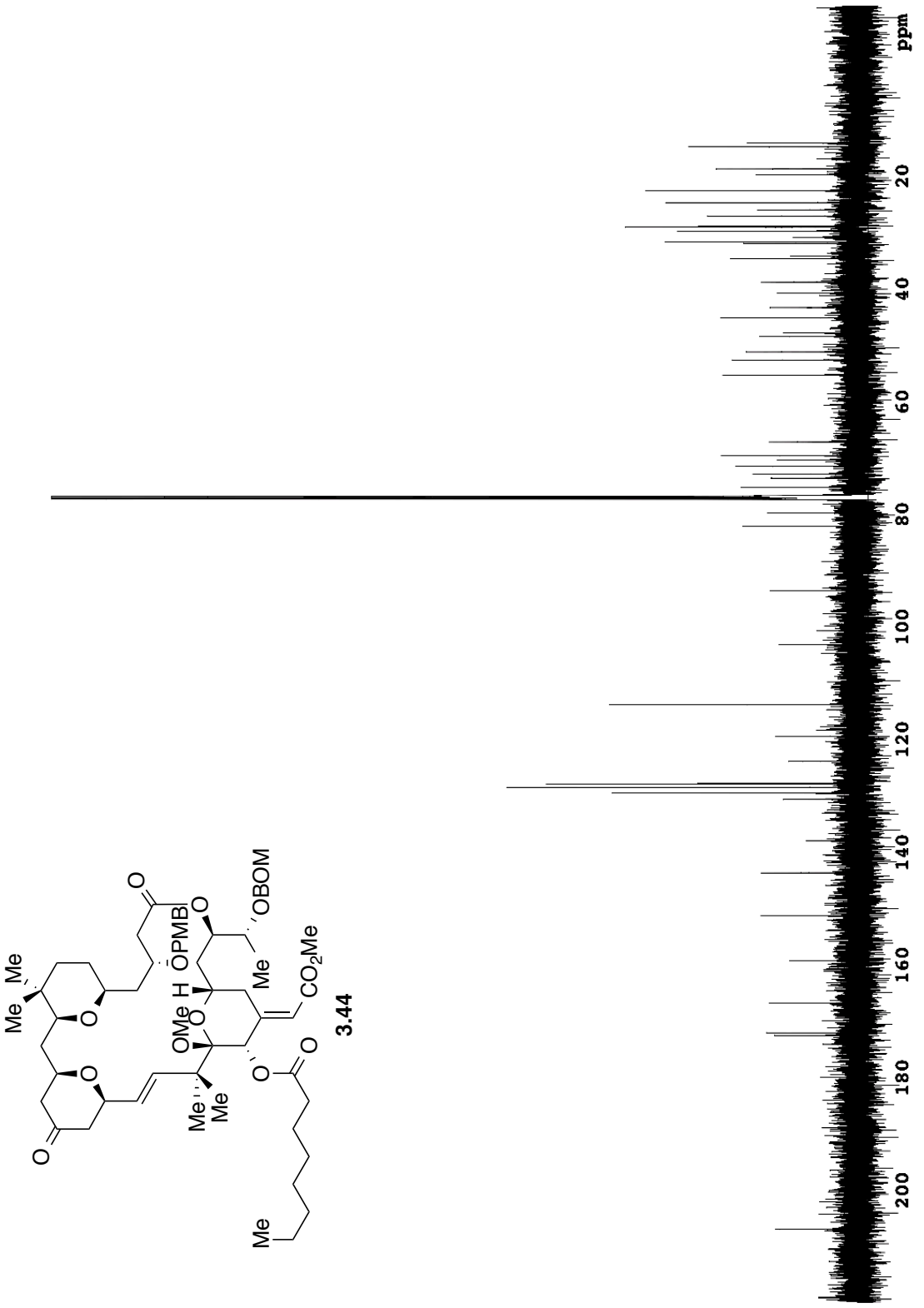

Me
Me
O
O
OMe
H
OPMB
O
OTBS
CHO
Me
Me
CO2Me
OBOM
O
O
Me
3.42
200
180
160
140
120
100
80
60
40
20
ppm

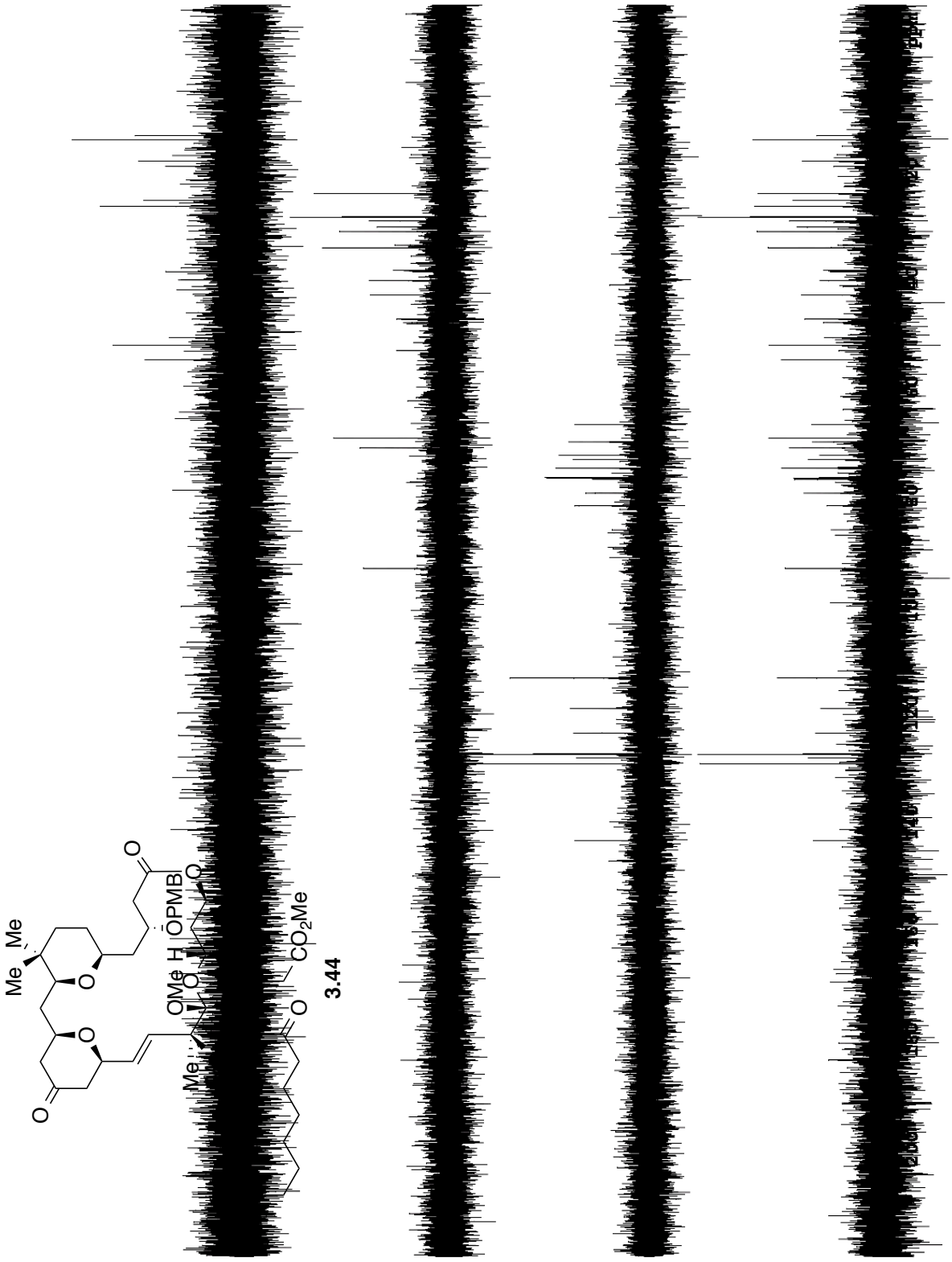

Me
Me
O
O
OMe
H
OPMB
O
O
Me
Me
O
O
Me
Me
OBOM
CO2Me
O
Me
3.43
ppm
1
2
3
4
5
6
7
8
9


Me
Me
O
O
O
OMe
H
OPMB
O
O
Me
Me
O
O
Me
Me
OBOM
CO2Me
O
Me
3.43
ppm
200
180
160
140
120
100
80
60
40
20


Me
Me
O
O
OMe
H
OPMB
O
Me
OBOM
CO2Me
O
O
Me
3.43
ppm
20
40
60
80
100
120
140
160
180
200


Me
Me
O
O
O
OMe
H
OPMB
O
OBOM
Me
Me
Me
Me
O
O
O
CO2Me
Me
3.44
ppm
1
2
3
4
5
6
7
8
9


Me
Me
O
O
O
OMe
H
OPMB
O
O
Me
Me
Me
Me
OBOM
CO2Me
O
O
Me
3.44
ppm
20
40
60
80
100
120
140
160
180
200


Me
Me
O
O
O
OMe
H
OPMB
O
O
Me
O
CO2Me
3.44